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BULLETIN OF
THE NEW YORK ACADEMY
OF MEDICINE

NOVEMBER, 1942

MARIHUANA*

ROGER ADAMS

Professor of Chemistry, University of Illinois

Harvey Lecture, February 19, 1942

THE facts on marihuana which I shall present to you this evening comprise the results of the coöperative efforts of three laboratories—the chemical investigations at the University of Illinois, the pharmacology at Cornell Medical College under the direction of Dr. S. Loewe, and the clinical experiments at Welfare Island Hospital under the auspices of the Mayor's Committee on Marihuana and under the immediate direction of Dr. Samuel Allentuck. All three laboratories acquired their supplies of raw materials from Dr. H. J. Wollner of the Narcotics Laboratory of the Treasury Department, and received much encouragement and stimulation from him. Dr. J. R. Matchett of the same laboratory contributed significantly to the general problem by devising a method whereby a fraction of the red oil of hemp, containing a very high concentration of the active principle, could be obtained.

Cannabis sativa, more commonly called hemp, is of peculiar interest. It has been known for thousands of years as a product of commerce; the fiber of the plant for clothing and rope, the seeds for the oil they

* From the Noyes Chemical Laboratory, University of Illinois, the Department of Pharmacology of Cornell University Medical College and the Welfare Island Hospital.

contain. The presence of an intoxicating principle in the resin of the plant has also long been recognized, since the physiological action of hemp preparations is mentioned in some of the earliest records available and is described in the first medical treatises. In the last two thousand years over four hundred articles have been published describing its intoxicating characteristics and the effects on humans.[1]

The preparation of the hemp for consumption as an intoxicant varies in different countries and consequently several names, such as marihuana, charas, ganja, hashish, and others, have been adopted, each in a definite locality. It is significant that the same characteristic phenomenon from any of these products is observed in man when the doses are equated.

The clarification of the chemical and medical aspects of hemp extracts has been extraordinarily slow for a material known as long and used as frequently as marihuana. The reasons have been several—the failure of chemists to isolate a pure active principle, the unsuccessful attempts of the pharmacologist to find an animal test which paralleled the activity in humans, and finally the lack of controlled clinical experiments.

The recorded medical literature is most confusing. The reports are contradictory, and the description of the drug varies from one which is habit-forming and which with constant use is as harmful to the system as morphine, to one which is almost completely innocuous with a stimulation not far remote from that of alcohol. Spread of the use of marihuana in the United States has been due in part to its ready availability, since hemp grows wild in countless places all over the country. Newspaper articles have described marihuana smoking among school children, and magazine articles have carried vivid accounts of activities encountered in tea-pads—dens where marihuana is enjoyed by devotees. Marihuana has been accredited with precipitating premeditated criminal acts, of lowering morals and releasing inhibitions, and of serving as a stepping-stone to other drug addictions such as the use of heroin. Furthermore, the claim has been made that continued use of marihuana produces mental deterioration. A "Marihuana Bill" was passed by the United States Congress in 1934. For purposes of administration marihuana is defined essentially as any part of the hemp plant or extract therefrom which induces somatic and psychic changes in man. The regulations and penalties in the bill for use and distribution

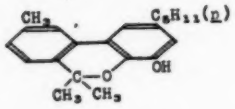
of marihuana are as rigid as those imposed for the use and sale of morphine.

In the investigations which are to be described this evening, more quantitative data on the chemistry, pharmacology, and clinical aspects of marihuana have been gathered than hitherto have been available and present a foundation for the eventual complete understanding of this interesting natural product. The chemical investigations, an excellent résumé of which up to 1938 has been published by Blatt,[2] will be discussed first. Practically all of the chemical experiments which have been reported were performed on the resin present in hemp from various sources and the same general procedure for isolating the resin has been used by all investigators in this field. After extraction with an organic solvent, filtration of the solution thus obtained, removal of the solvent and vacuum distillation of the residue, a highly viscous, physiologically active oil results, red in color and boiling over a wide range. Fractionation of the oil leads to the concentration of the active components in the portion boiling from 180-190° (1 mm.) which is commonly known as "purified red oil." This purified red oil usually used in the chemical studies has been shown definitely to be a welter of closely related substances which are very difficult to separate from each other and which occur in varying proportions dependent on the source of the hemp.

Between 1840 and 1895, most of the chemical investigations consisted in attempts to discover tests which would provide means of identifying a hemp extract. Numerous color reactions were reported, only one of which has received frequent application and until recently general acceptance. This is the so-called alkaline Beam test which is the purple color produced by treatment of a hemp extract with 5 per cent methanolic potassium hydroxide.[3] From our chemical results and from an extensive investigation of agronomic varieties of hemp by Dr. Matchett[4] it can be concluded that this test is not indicative of a substance with marihuana activity.

In 1895 Wood, Spivey, and Easterfield[5] were able to isolate from "purified red oil" by means of a treatment with acetic anhydride, a crystalline acetate, which was removed from the residual oil and which could be purified in the normal way. Hydrolysis of this pure acetate resulted in a homogeneous viscous oil which these investigators called cannabinol. Until about a decade ago, when the fact was shown to be erroneous, cannabinol was accepted as the active principle of hemp.

CHART I



(Cannabinol, Cahn)

Cannabinol	M.P. °C.	Cannabidiol	M.P. °C.
$C_{21}H_{26}O_2$	75–76	$C_{21}H_{30}O_2$	66–67
p-Nitrobenzoate	165–166	*bis*-3,5-Dinitrobenzoate	106–107
m-Nitrobenzenesulfonate	127–129	*bis*-*m*-Nitrobenzenesulfonate	119–120
Acetate	76–77	Oil	
3,5-Dinitrophenylurethan	220–222	Oil	
Optically inactive		$[\alpha]^{27}D$—125° (ethanol)	
No alkaline Beam test		Superb alkaline Beam test	
No marihuana activity		No marihuana activity	

These same investigators performed preliminary experiments on the structure of the cannabinol molecule. Further work on its chemistry was impeded by the fact that in spite of many attempts in different laboratories the isolation of cannabinol was not repeated until 1932. At that time Cahn[6], an English chemist, again obtained this compound and completed a series of brilliant researches from the results of which he was able to establish the skeleton and the substituents in the molecule but was unsuccessful in determining the orientation of all the groups.

It was with this background that the chemical experiments were begun at the University of Illinois.* Attempts to isolate cannabinol from the purified red oil of Minnesota wild hemp by the procedure described by Cahn failed. Consequently, attention was turned to attempts to isolate a phenolic product, the presence of which was established by qualitative tests. Of the numerous reagents employed, 3,5-dinitrobenzoyl chloride reacted to give a crystalline compound which was readily removed from the residual oil and purified. It proved to be a *bis*-ester, the hydrolysis of which by an appropriate method gave a new substance which was termed "cannabidiol" because of the presence of two phenolic groups.[7] It was isolated first as an oil but eventually was obtained as a crystalline solid. By developing a new procedure as a substitute for Cahn's method the isolation of cannabinol from this same oil was also accomplished, and for the first time cannabinol was induced to crystal-

* The experimental work of the series of researches at the University of Illinois was performed by the following students: B. R. Baker, C. K. Cain, J. H. Clark, Madison Hunt, Charles F. Jelinek, W. D. McPhee, D. C. Pease, C. M. Smith, R. B. Wearn and Hans Wolff.

lize.[8*] The properties of cannabinol and cannabidiol are compared in Chart I. Cahn's proposed formula for cannabinol is shown; his evidence for this structure was conclusive except for the positions of the hydroxyl and *n*-amyl groups.

The similarity in the empirical formulas of these two compounds is striking and led to the belief that these two sister substances must have structural formulas not too unrelated. The optical activity of cannabidiol suggested immediately the probability of partial hydrogenation of one aromatic nucleus in cannabinol and indeed the left one, since cannabidiol contains phenolic groups.

Although cannabidiol, like cannabinol, is physiologically inactive, the study of its structure and its reactions was most revealing. The results served to determine completely the structure of cannabinol and led to the formation of tetrahydrocannabinols, products of high marihuana potency which are probably active principles in the red oil of hemp.

The complicated and extensive chemical investigations on the structure of cannabidiol, on the synthesis of cannabinol, and on the preparation of tetrahydrocannabinol and synthetic analogs, will be presented in very brief form and only the more significant facts will be mentioned. The structure of cannabidiol[10] will be considered first with the pertinent reactions given in logical rather than chronological order.

Typical color tests indicated a phenol group, and formation of *bis*-esters and ethers the probable presence of two such groups. Catalytic reduction resulted in the absorption of two moles of hydrogen with formation of a molecule which still retained the two phenolic groups, thus leading to the deduction that two aliphatic double bonds were present. Pyrolysis of cannabidiol with pyridine hydrochloride caused cleavage into *p*-cymene and olivetol (1,3-dihydroxy-5-*n*-amylbenzene) both of which were identified by comparison with authentic samples. This is convincing evidence that cannabidiol is composed of dihydrocymyl and olivetol residues. The positions of the linkage between these residues were determined next. Cannabidiol was first reduced to tetrahydrocannabidiol and then oxidized; menthane carboxylic acid was isolated, identical with a specimen obtained by synthesis from l-menthol,

* Cannabidiol and cannabinol are the only pure compounds related by structure to the active constituents which have been isolated from hemp extracts. Claims have been made for the isolation of other compounds or their derivatives, but no detailed information is available and the results require confirmation.[9]

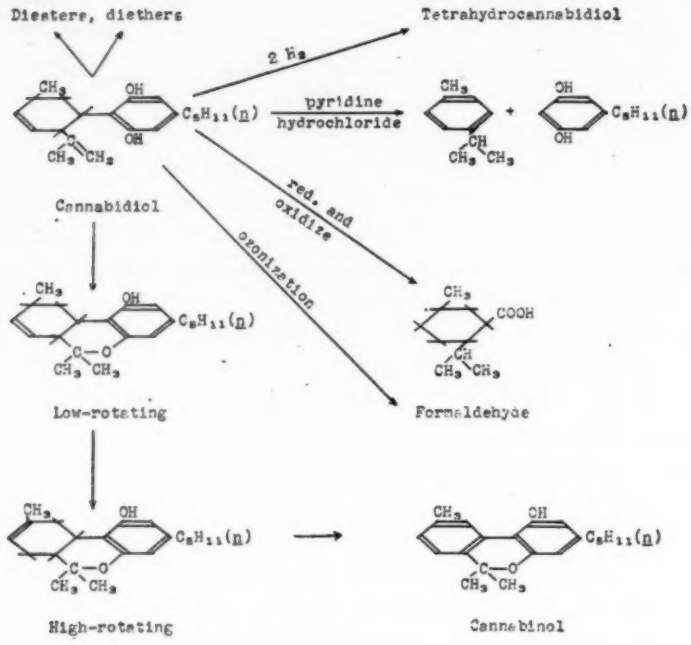


CHART II

thus demonstrating that the attachment of the dihydrocymyl residue was adjacent to the isopropyl grouping. From a comparison of the absorption spectra of various amyl resorcinols and of cannabidiol and its reduction product, the dihydrocymyl was postulated as being attached to the olivetol between the hydroxyl groups. Direct chemical proof of this was accomplished by conversion of cannabidiol with acidic reagents to tetrahydrocannabinol which, upon dehydrogenation, gave cannabinol. Cannabinol was shown to contain the linkage between the hydroxyls by synthesis of this molecule by an unequivocal method.

The structure of the cannabidiol molecule was thus established except for the orientation of the two aliphatic double bonds in the dihydrocymyl residue. One of these proved to be terminal, since ozonization of cannabidiol gave formaldehyde. This information, along with the fact that tetrahydrocannabinol was produced from cannabidiol through closure of a pyran ring, left no doubt that this terminal double

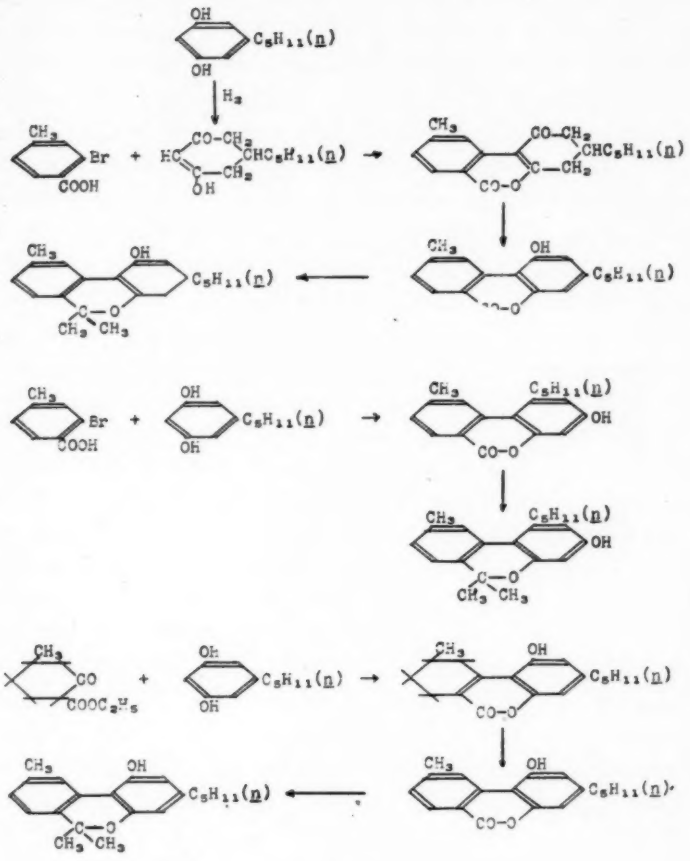


CHART III

bond must be present as an isopropenyl group. The location of the second double bond was determined only by indirect means. Since the arguments are rather involved, they will be omitted here and merely the positions assigned for the double bonds will be given. The tetrahydrocannabinol obtained in the isomerization of cannabidiol, varied in rotation dependent on the reagent used. Apparently two forms exist which were isolated as low-rotating and high-rotating isomers. In the low-rotating tetrahydrocannabinol, which presumably has the double bond in the same position as in cannabidiol, the double bond was deduced

to be in the γ,δ-position between the unsubstituted carbons; in the high-rotating isomer, the double bond is probably substituted in the γ,δ-position which includes the ring carbon holding the methyl group.

The establishment of olivetol as a cleavage product of cannabidiol revealed the probable orientation of the hydroxyl and *n*-amyl groups in cannabinol. This supposition was proven correct by the synthesis of cannabinol by two different methods[11] as shown in Chart III. The syntheses served also to prove that the position of the linkage of the two benzene rings is between the hydroxyl groups and thus confirmed a similar attachment of the rings in cannabidiol.

2-Bromo-4-methylbenzoic acid and dihydroolivetol condensed in the presence of alkali and a copper salt to 1-keto-3-*n*-amyl-9-methyl-1,2,3,4-tetrahydro-6-dibenzopyrone; dehydrogenation gave 1-hydroxy-3-*n*-amyl-9-methyl-6-dibenzopyrone which, with excess of methylmagnesium iodide, gave cannabinol, (1-hydroxy-3-*n*-amyl-6,6,9-trimethyl-6-dibenzopyran). An analogous condensation of 2-bromo-4-methylbenzoic acid with olivetol, followed by treatment with methylmagnesium iodide, gave the isomeric cannabinol with the linkage between an hydroxyl and the *n*-amyl group. The second method consisted in condensation of ethyl 5-methylcyclohexanone-2-carboxylate with olivetol in the presence of phosphorous oxychloride to give 1-hydroxy-3-*n*-amyl-9-methyl-7,8,9,10-tetrahydro-6-dibenzopyrone. Dehydrogenation to the corresponding 1-hydroxy-3-*n*-amyl-9-methyl-6-dibenzopyrone followed by treatment with methylmagnesium iodide gave cannabinol.

The disappointment accompanying the discovery that cannabidiol had no marihuana activity was more than compensated by the observation that both the low-rotating and high-rotating tetrahydrocannabinols possess very marked marihuana potency.[12] These two substances, which were high-boiling oils, have not yet been induced to crystallize. All attempts to isolate solid crystalline derivatives have failed. The isomerization of cannabidiol required intensive study before procedures were found which resulted in products of constant rotation. Apparently, without very specific conditions, mixtures of low- and high-rotating forms are obtained which cannot be converted readily to a product of maximum rotation. Tetrahydrocannabinol of constant rotation $[\alpha]^{27}$ D–265° can be produced conveniently from cannabidiol merely by heating the latter in benzene solution with a little toluene sulfonic acid until the reaction mixture exhibits no alkaline Beam test. This product was the

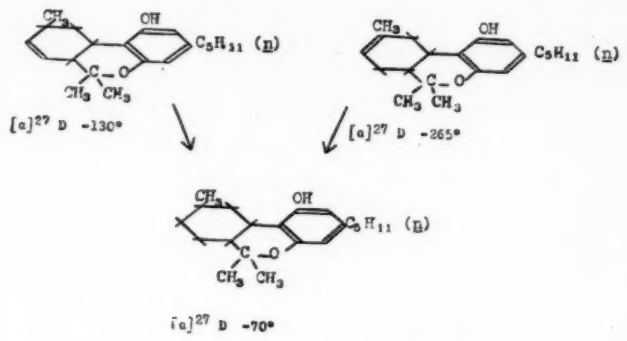


CHART IV

principal one selected for pharmacological and clinical investigation.

The acetates of these tetrahydrocannabinols also had marihuana potency though less than that of the unacetylated compounds. By catalytic reduction all of the tetrahydrocannabinols of varying optical activity gave a hexahydrocannabinol of essentially the same optical activity. This product was physiologically active though less so than any of the tetrahydrocannabinols from which it was derived.

The typical marihuana activity manifested by the isomeric tetrahydrocannabinols constitutes ponderable evidence that the activity of the plant itself, and of extracts prepared therefrom, is due in large part to one or the other of these compounds, or both, and possibly also to their stereoisomers, of which a number are possible. Confirmation of this supposition is available from an investigation by Wollner, Matchett, Levine and Loewe,[13] the results of which were published just recently. These authors have described the isolation from acetylated red oil of a tetrahydrocannabinol acetate of potency greater than that of either of the tetrahydrocannabinols prepared by isomerization of cannabidiol. Partition of the acetylated red oil was accomplished by selective adsorption. Silica gel removed cannabidiol diacetate and unknown material from a benzene solution of the mixture; alumina adsorbed substances of lower rotation by two passages, first in carbon tetrachloride, then in pentane solution. The product was judged to be stereochemically homogeneous by failure to effect further separation through selective adsorption or by careful fractional distillation in a specially designed high-vacuum fractionating column. Hydrolysis of the acetate yielded a tetrahydro-

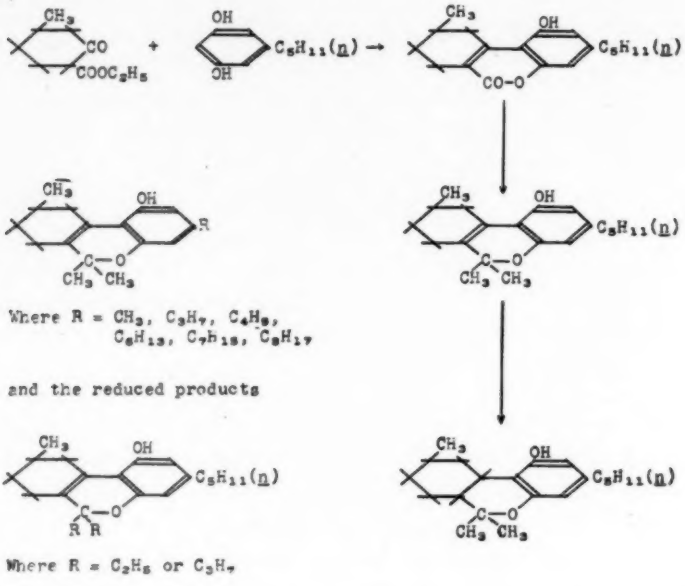


CHART V

cannabinol whose structure was identified through analysis and dehydrogenation to cannabinol. Its potency was similar to the products prepared by isomerization of cannabidiol. It would appear that rearrangement occurred during hydrolysis, since reacetylation failed to restore either the optical rotation or physiological potency to the original value.

With the discovery of the character of substances which possess marihuana activity, attention was directed next to attempts to synthesize compounds of similar activity. A very satisfactory procedure was devised for obtaining an isomer of the natural tetrahydrocannabinol with the double bond conjugated to the benzene ring. It consisted in the condensation of ethyl 5-methylcyclohexanone-2-carboxylate with olivetol to give 1-hydroxy-3-*n*-amyl-9-methyl-7,8,9,10-tetrahydro-6-dibenzopyrone which, with excess methylmagnesium iodide, yielded 1-hydroxy-3-*n*-amyl-6,6,9-trimethyl-7,8,9,10-tetrahydro-6-dibenzopyran.[14] The product proved to have marihuana activity though only about one-tenth that of its isomer, natural tetrahydrocannabinol. A series of several closely related synthetic compounds was then prepared by the same

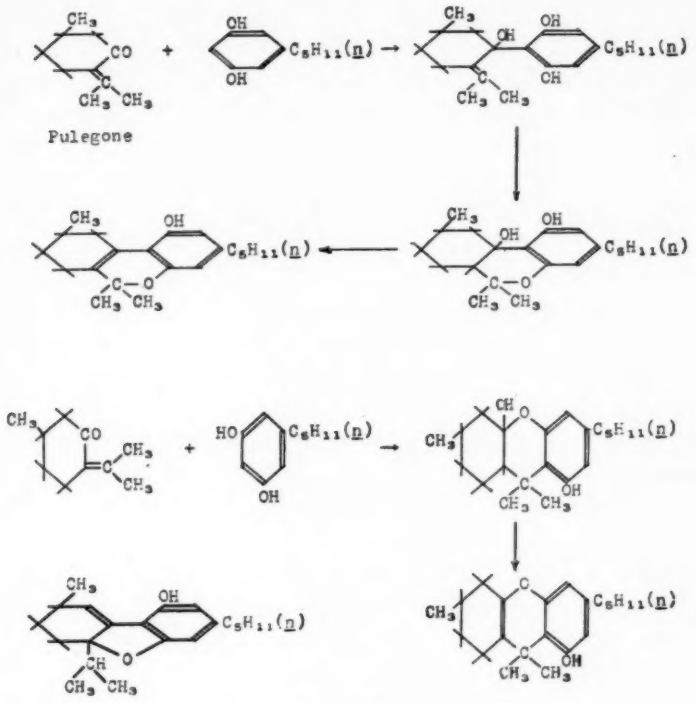


CHART VI

Homologs were prepared in which the *n*-amyl group was substituted by C_3H_7, C_4H_9, C_6H_{13}, C_7H_{15}, C_8H_{17}, C_9H_{19}; the reduction product of each was also synthesized.

procedure using the same keto ester but homologs of olivetol.[15] All of these products were reduced to the corresponding hexahydro compounds.[16] The synthetic tetrahydrocannabinol was also modified by replacing the 6-methyl groups by ethyl and propyl groups.[17] (Chart V.)

A provisional synthesis designed to obtain an optically active tetrahydrocannabinol was sought in the condensation of pulegone and olivetol.[17,18] In the presence of phosphorous oxychloride, a product which analyzes for, and has the properties of tetrahydrocannabinol is formed. A possible mechanism by which such a reaction might take place is shown in Chart VI. The purity of the final product is by no means established. There is a possibility of contaminants formed by condensation of the two reactants to give a partially hydrogenated xanthane

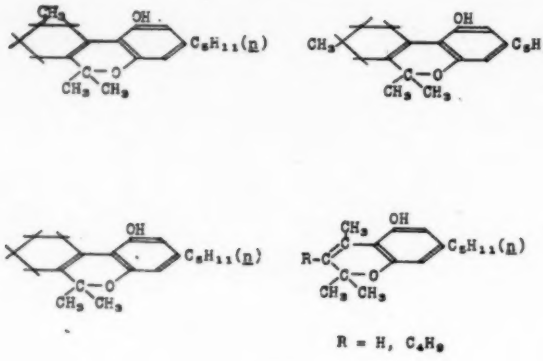


CHART VII

or a tetrahydrodibenzofuran, isomeric with tetrahydrocannabinol. Homologs in which the olivetol portion was substituted by other 1,3-dihydroxy-5-alkylbenzenes were synthesized.[16] The reduction product of each was prepared.

Finally in Chart VII are shown molecules in which the left-hand ring has been modified by removal or by change in the position of the methyl group.[17]

The pharmacological studies on hemp extracts have been equally as meager as the chemical investigations and in the long history of *Cannabis* preparations only three tests have been reported. Liataud[19] in 1844 observed that motor incoördination in the dog was a characteristic effect induced by marihuana preparations; Fraenkel[20] in 1903 interpreted this incoördination as a cataleptic condition. This was followed in 1928 by the observations of Gayer[21] that in various animals such as cats, rabbits, or dogs, intravenous injection of marihuana preparations in acetone solution induced corneal anesthesia which was characteristic of active fractions of the resin. In 1937, Munch and Mantz[22] reported no unequivocal effects when *Cannabis* preparations were administered to albino mice. On the other hand, Loewe[23] noted a definite increased depressant action when treated mice were given a hypnotic of the barbiturate series. Pernocton, butyl-bromoallyl barbituric acid, gave the greatest enhancement of any of the drugs tested.

The Gayer corneal anesthesia test was developed further by Marx and Eckhardt[24] but neither these investigators nor Gayer himself went further than to designate that the corneal reflex was normal or abnor-

mal. The corneal response was ascertained by tapping with von Frey hairs. Walton[25] took steps in a quantitative direction by counting the number of responses occasioned by tapping the cornea a given number of times and by plotting the results over the whole duration of the effect. By thus locating a definite maximum in the areflexia-versus-time curve of each experiment, quantitative comparisons were made possible by determining the ratios of doses producing equal maxima in different animals. Loewe[26] developed this procedure further by application of his method of "Bioassay by Approximation" to overcome as far as possible the large intra-individual and the large group variabilities which seem to be inherent in the reaction of all types of animals to marihuana preparations. His study of the Gayer test applied to the behavior of rabbits as test animals showed not only great inter-individual variations in sensitivity but also enormous intra-individual variations in the same animal. Using the same animal repeatedly, this investigator found a consistent decrease in sensitivity to one and the same dose. Therefore, even though the method of approximation was applied, the values of potency obtained by this method are not suitable for anything but qualitative purposes. Moreover, they do not parallel the dog-ataxia potencies of the same preparations, the divergence sometimes being tenfold. This indicates either that the Gayer test is not conclusive for quantitative measurements or else that an active principle other than that disclosed by the dog-ataxia test is present in red oil.

The "mouse sleep prolongation test" may be dismissed with merely a brief discussion. The *Cannabis* preparations, usually red oil, were administered by stomach tube. After a definite time, pernocton was injected intravenously at a level just above the threshold of hypnotic action. The synergistic effect of the *Cannabis* was measured by the period of suppression of the righting reflex averaged over all the animals of a single-dose group. This effect, though typical of red oil, could not be duplicated with the natural or synthetic tetrahydrocannabinols which had been shown definitely to have physiological activity in man. Pure cannabidiol, which is devoid of the marihuana effect upon man, showed the highest potency in this test and consequently the action from the red oil probably is due to its cannabidiol content.

The determination of the cataleptic condition in dogs reported by Fraenkel, as accepted in former editions of the United States Pharmacopoeia for bioassay of *Cannabis* extracts, was developed further by

Walton[25] to attempt to make the effect the basis of a quantitative test. Six arbitrary stages of intensity of effects were recognized; first a slight depression; second, a barely detectable ataxia; third, an obvious ataxia; fourth, a marked ataxia in which the animal frequently pitches forward and barely catches itself; fifth, inability to stand alone; sixth, inability to rise and plunge about. The ataxia is chiefly a static one and is manifested particularly by swaying movements. Intravenous doses of red oil dissolved in acetone always acted within half an hour. A similar intensity of effect by oral administration required five to seven times the dose. Walton employed for evaluating potency the procedure used in most bioassay methods, the comparison of test and standard doses of equal intensity of effect. By comparing the results at various levels of dosage and by repeating the procedure a considerable number of times, he obtained more accurate results than had hitherto been reported. About eight trials with the unknown on the same dog, calibrated in about six trials with the standard, were used. A single assay required about three weeks or more for completion.

Loewe applied his principle of "Bioassay by Approximation" to the ataxia test and has thus been able to obtain a more decisive method for comparison of active products. The procedure aims at obtaining from each one of an adequate number of calibrated dogs, several figures of comparison of a test dose with the calibration doses. These figures represent ratios of doses, the response to which is not quantitatively the same. They are used to approximate the true potency value from both sides. At the same time the degree of overlapping marks the range of variation and gives an idea of the inherent inaccuracy. An entire assay may in this way be performed in a single day and highly consistent results may be obtained. The maximum order of accuracy is 10 per cent since this is the minimum of variation in the response of the same animal at different times. Since parallelism between the results of the dog-ataxia tests and the effects of the different preparations on humans has been established, it may be concluded that the ataxia method as developed by Loewe represents a reliable index of potency.

Using this procedure as just outlined, the comparison of results on the various natural and synthetic products will be presented in charts. Each value given represents the result obtained by the use of several dogs; three or four in the case of low potency materials, ten to twenty or more for substances of higher potency.

CHART VIII

R=*n*-alkyl	Potency Tetrahydro	Hexahydro
CH_3	0.16 ± 0.03	below 0.04
C_3H_7	.40 ± .08	0.26 ± .04
C_4H_9	.37 ± .12	.37 ± .06
C_5H_{11}	standard 1.00	.51 ± .08
C_6H_{13}	1.82 ± 0.40	1.86 ± .37
C_7H_{15}	1.05 ± .15	0.83 ± .13
C_8H_{17}	0.66 ± .13	.24 ± .06

Tetrahydrocannabinol $[\alpha]^{22}D$	Parke, Davis and Company	
−265° 7.3 ± 0.89	Fluid Extract	0.060
−260° 7.8 ± .78	American Fluid Extracts	
−240° 7.6 ± 1.1	thirty to forty different	
−165° 9.3 ± 2.9	samples varied in	
−160° 8.23 ± 2.17	potency	.003–0.130
−126° 6.5 ± 0.65	majority varied	.019– .052
Hexahydrocannabinol	Purified red oil	1.24
−70° 3.0 ± 0.43	Highly purified red oil	
	(Matchett)	4.33

In Chart VIII is shown a comparison of the potencies of the series of products analogous to synthetic tetrahydrocannabinol. The latter was adopted as a standard. The corresponding hexahydro derivatives were also tested. It is observed that the modification of the alkyl group results in a gradual increase in activity with increase in size until a maximum is reached at the *n*-hexyl derivative. The point of maximum potency is the same in the hexahydro compounds, though all, with the exception of the *n*-hexyl, exhibit a decreased effect. There is also presented the potencies of tetrahydrocannabinols of different optical rotations all derived from cannabidiol, the potency of an average purified red oil and of a highly active portion of red oil obtained from it by extraordinarily careful fractionation. The increased activity of the optically active natural tetrahydrocannabinols is striking. The commercial cannabis fluid extracts are of very low and variable potency.

In Chart IX, the potency of products of questionable purity produced from pulegone and various olivetol homologs, together with their hy-

CHART IX

Pulegone Condensation Products

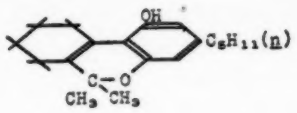
R = *n*-alkyl	Potency	
	Original products	Reduced products
C_3H_7	below 0.23	< 0.20
C_4H_9	0.25 ± .10	< .15
C_8H_{11}	.58 ± .12	.64 ± 0.10
C_6H_{13}	1.22 ± .12	.78 ± .22
C_7H_{15}	1.15 ± .15	.83 ± .17
C_8H_{17}	1.37 ± .25	below .25
C_9H_{19}	below .20	.....

drogenated derivatives are shown. The maximum potency appears in the *n*-octyl molecule, and the values for the *n*-heptyl and *n*-octyl derivatives exceed those of the corresponding products synthesized by an unequivocal procedure.

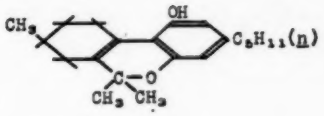
Finally in Chart X, the activities of other analogs are shown. Each has an activity less than that of the molecule possessing methyl groups in the 6,6,9-positions.

Recognizing that the indulgence in marihuana in New York City has constituted a growing problem of major consequence involving psychiatric, medical, legal, sociological and civic aspects, a clinical study was undertaken by a committee appointed by Mayor LaGuardia and supported by funds allotted by several foundations. The primary objectives were the determination of the mental and physical actions of marihuana on the kind of person resorting to its use and the consequent social implications. Dr. Samuel Allentuck directed the clinical studies in a unit of the Welfare Island Hospital and the facts I am presenting have been summarized from his report on the results.

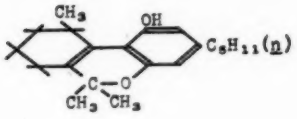
An orientation group, the members of which were subjected to numerous and varied procedures, was used to determine precisely which tests would best lend themselves to the solution of the specific problems. From the results a program was established which consisted in a systematic study of a group of seventy-seven subjects ranging in age from 21 to 45 years and from borderline to superior in intelligence, all of them voluntary recruits from one prison population. About half had used marihuana previously. After a physical, neurological, and psychiatric examination, they were placed in one or more of five categories as to personality types—normal, antisocial, autistic, cyclothymic and epileptic. Each individual before and during the period of action of



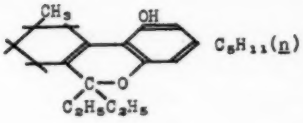
0.126±0.05



0.137±0.01



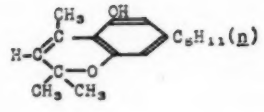
0.25±0.05



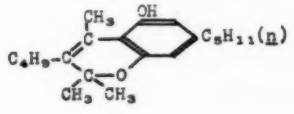
0.12±0.024

$(C_3H_7)(C_3H_7)$

0.04±0.01



0.033±0.010



0.04±0.01

CHART X

marihuana was interviewed at regular intervals throughout the day by various members of the staff. Possible subjective and objective phenomena resulting from use of the drug were discussed and elaborated upon in detail. Introspective reports were obtained in the absence of the drug, under pseudo-stimulation with placebos and during intoxication with marihuana or allied synthetics; analysis of the data was based on the most frequently mentioned phenomena. The patients were also subjected to periodic tests for blood pressure or pulse changes, pupillary changes, urinalyses, blood chemistries, hematological surveys, basal metabolic rates, electrocardiograms, arterial and venous pressure tracings and vital capacities. In addition, psychological examinations before and during the intoxication periods were carried out, including a wide variety of psychophysical, psychomotor and clinical tests.

The marihuana was supplied in the form of a fluid concentrate which was desolvated and administered in the form of pills. Pure tetrahydrocannabinol was diluted with a little olive oil and placed in gelatin cap-

sules, holding 15 mg. of drug, the equivalent in physiological potency to one pill containing 300 mg. of crude solids from hemp. The 1-hydroxy-3-*n*-amyl-6,6,9-trimethyl-7,8,9,10-tetrahydro-6-dibenzopyran and its 3-*n*-hexyl homolog were administered in a similar manner using the equivalent doses which produced similar activity, namely, 120 mg. per capsule of the former and 60 mg. per capsule of the latter. It is significant that these relative amounts are practically identical with those observed by Loewe for obtaining identical effects in dog-ataxia tests.

The patients were started on two marihuana pills or on equated doses of tetrahydrocannabinol or the two synthetic analogs. The dose was increased by two pills at a time at intervals of two days unless toxic symptoms supervened. At the appearance of toxicity, the patient was returned to the physiological dose and this was increased one pill at a time. Thus the maximum tolerated dose for each individual was determined and at the same time approximately the threshold at which psychotic changes first appeared. Tetrahydrocannabinol and the synthetic compounds dissolved in olive oil were in some cases administered by intramuscular injection. Other clinical tests were made which involved intoxication from smoking marihuana cigarettes.

Barbiturates, cold showers and sweet candies were found to be efficacious in ameliorating any alarming physical or psychotic symptoms which developed following marihuana overdosage.

The detailed results of this carefully planned and executed clinical investigation, the first of its kind on record, must be left to the complete report when it is published. Merely the more significant findings which may prove of maximum value will be presented here. The crude drug in the form of concentrated marihuana extract, tetrahydrocannabinol derived from cannabidiol, and the two purely synthetic compounds, 1-hydroxy-3-*n*-amyl-6,6,9-trimethyl-7,8,9,10-tetrahydro-6-dibenzopyran and the corresponding 3-*n*-hexyl derivative elicited similar clinical and psychiatric phenomena upon the same subjects. The pharmacological action of these drugs somewhat resembles atropine and the psychiatric portrait, alcohol. The effects of marihuana do not vary qualitatively with the route of administration, whether ingested, injected or inhaled. By inhalation, however, they are more prompt in their appearance and disappearance; by ingestion they appear within one-half to one and one-half hours, reach their maximum in from three and one-half to five hours and disappear within seven hours.

The observed physical effects, one or more of which occur in each patient, are (a) elevation of the pulse rate, the increase being directly proportionate to the degree of intoxication; (b) elevation of the blood pressure; this varies with the individual and usually rises in direct proportion to the pulse; (c) injection of the conjunctival blood vessels which varies with the dose; (d) dilation of the pupils and sluggish reaction to light and in accommodation; vision for proximity, distance and color changes slightly; (e) circum oral tremors; tremulousness of the protruded tongue and the extremities; (f) dryness of the oral and pharyngeal mucous membranes; (g) increased frequency with decreased amplitude of thoracic respiratory movements; (h) ataxia; (i) hyperreflexia. The observed psychiatric effects are (a) apprehension and anxiety, (b) euphoria, (c) loquaciousness, (d) lowering of inhibitions, (e) hunger and thirst, (f) feeling of being "high," (g) uncontrollable bursts of laughter or giggles, (h) drowsiness, languor, lassitude and a pleasant feeling of fatigue.

Clinical tests revealed that marihuana produces no significant changes in basal metabolic rates, blood chemistry, hematological picture, liver function, kidney function or cardiac electrical conduction. Marihuana delays somewhat gastric and intestinal motility as gauged by the Carlson apparatus and x-ray studies; it produces definite increase in the frequency of the alpha wave in electroencephalographic recordings thus indicating increased relaxation.

Other observations of a more general character were recorded. Tolerance for marihuana may be produced by repeated administration of subtoxic doses over a prolonged period of time. Thus the same dose elicited progressively fewer and milder symptoms. Marihuana is unlike opium derivatives in that it does not give rise to a biological dependence accompanied by withdrawal symptoms. Neither does it establish a strong craving as exists in tobacco smoking or in alcoholic indulgence. Follow-up of the subjects has failed to establish existence of any craving for the product. Many of the unpleasant physical symptoms previously mentioned appear only as a result of the administration of excessive doses of drug. It is no more of an aphrodisiac than alcohol.

Since all the clinical experiments at Welfare Island were conducted on volunteer prisoners, it was desirable for completeness or perhaps to satisfy my curiosity to obtain some results on subjects in another social class. As a consequence, I have conducted a dozen or more experiments

using as test individuals chemists among whom were two members of the National Academy of Sciences and two high-ranking and very successful industrial chemists. In all cases very small doses, 15 mg. or 30 mg. of tetrahydrocannabir ' were administered about one hour before dinner. Each individual reac d differently with the possible exception of the observed stimulation of the appetite. They all recognized an intoxication which they described as in general like, but in detail different from that induced by alcohol. Thus, one industrial chemist who shows no outward change under the influence of alcohol, reported essentially no effect from 15 mg. except a mild stimulation of his desire for food. A 30 mg.-dose, however, to this same individual had a pronounced effect. Though he noticed no particular craving for food before dinner, as soon as he started eating he became particularly hungry and consumed a very large meal. He felt intoxicated and dissociated from his normal self, had a feeling of heaviness in his head and legs and reported a fogginess which he described as the inability to focus his eyes on more than a single object at a time. Since this man desired to get the effect of distorted time and space which is recorded as a frequent phenomenon associated with the marihuana user, he tried it a third time, taking 45 mg. The result was a ravenous hunger which was not satisfied after eating the equivalent of two hearty meals. A marked hypergeusia was also noted. The same fogginess appeared and heaviness in head and legs. During the conversation which took place among his five associates at the dinner table, he was able to comprehend a question but by the time the answer was given, which was immediately, he couldn't remember the question. In spite of the intoxication with the resulting phenomena, this subject had no difficulty in holding his own and then some in a poker game composed of expert players.

A second industrialist took 15 mg. at 5:00 o'clock in the afternoon and felt the first effects about 6:30 when he lost coördination in his fingers to the extent that he had to stop playing the violin, which he was doing at the time. Shortly thereafter he developed a tremendous appetite which was, if anything, sharpened by eating an enormous dinner and popcorn all through the evening. He had a mild lift about like a cocktail or two on an empty stomach and this and the hunger left about 11:00 o'clock.

A chemistry professor who took 30 mg. had a mildly increased appetite and reported feeling a bit fuzzy during his dinner, which resulted

in difficulty in comprehending what his associates were saying. This was followed by sleepiness and lassitude until the effects of the drug disappeared two hours afterwards. The stimulation was only slight, which paralleled the effect of alcohol upon this man.

A fourth subject of high standing in university circles wrote me in detail concerning his experience. I am quoting from his letter received two days after the experiment.

"This is to report to you on the outcome of my trip conducted under the powerful guidance of the marihuana drops. I would be interested some time to know just how much of what specific material you gave me, but there is no question but that it gave me a most terrific wallop. In brief:

"5:20 P.M. Took two capsules, went for short swim, had a highball and began to feel something beyond the mild glow from the drink about 6:15. By 6:30 felt bouncy in the knees, a little gay and foolish.

"6:00-8:30 P.M. Very much in the fog. Had alternate waves of hilarity and depression. Sat in smoking compartment looking at myself in the mirror, writing notes on the experiment, and feeling very silly and stupid. Would feel the onset of a surge of hilarity and then break into a raucous, rippling laugh. This gayety was not particularly pleasant, however, for throughout I felt wholly dissociated from myself, knew that I was at the mercy of the drug, and greatly resented this lack of control. The feeling was very different from that of being at one or another stage of intoxication, for I looked perfectly clear and normal and I could stand erect without swaying and execute motions with considerable precision. I could not, to my annoyance and as I was well aware, speak or write or think coherently. This bothered me particularly in the waves of depression, when my lips would feel very parched and salty and I would long to break the spell and regain my own consciousness. A very pressing and persistent sensation was that of extreme hunger, but I had sense enough to wait until the laughing spells were under control before going into the diner.

"Here are a few excerpts from the log: '7:20. Not so good; for a few minutes I sat and looked at myself in a silly way. . . . This is *me* again. I very suddenly snapped out of it and am struggling back to normal. Lips are very dry. Maybe I'm not *quite* out of it. . . . The above is true. I am writing here in a serious vein—but quick, I must write that a minute or two ago I was sitting here in the men's lounge giggling at

myself in the mirror and saying: This stuff does make you feel pretty gay (gay in the neese). Isn't that the damndest thing? [I knew the spelling was wrong but couldn't right it.] . . . 7:42. Yes, snapping out again. I just had a most jubilant laugh and feel another coming along. 7:45, not feeling laughy, feel like hell. This is really awful stuff. . . . 8:03. I feel like a fool. Lips bad. Want water, but I am terribly hungry and wish the experiment were over. I am thinking very much of eating, for I am very hungry. . . . 8:09. Nearly came out of it. It is awful. Helpless, awful feeling. Over, over, when will it be over? When can I eat? . . . 8:13. A fellow just came in to shave. Why now? Why not at this time of the evening EAT. . . . ha, ha. Now I have been silly. Looked silly. . . . ha, ha. Of all places to have this—the train. Bad, bad. Oh I feel like hell, salty lips . . .'

"At 8:30 I devoured an enormous steak dinner with great rapidity and thoroughness, and left no trace of any of the fixings, even though I ordinarily do not eat ripe olives or salad, and although ordinary delicacy would keep me somewhat below the ten crackers I had with my cheese. The food tasted no better or worse than usual, and I had a dissociated feeling that my mouth was a purely mechanical guide for all that came its way, and wondered if mine was not very much the same as the 'appetite' of a cat.

"At 9:00 I felt myself coming out of the spell, and again at 9:15 I felt sane for a minute or two. A little later the sane periods began to predominate, and by 10 P.M. I was back again in control and could sit down and write out the details of a new natural product synthesis.

"Thus ended the trip. I didn't sleep too well or too poorly, and the next morning I felt O.K. and had no hang-over.

"It was an interesting experiment, but I can't write too enthusiastic an endorsement for this drug you fellows are synthesizing. The feeling of well-being would not, in my estimation, equal that from about three highballs, and the penalty seemed to me to be pretty severe. The outstanding impressions were the feeling of detachment from myself and the extreme hunger. Are these both associated with the same part of the molecule? If not, you might hydrogenate out some of the bad effects and thereby obtain a wonderful aperitif."

After the Welfare Island study of every phase of the action of marihuana and the synthetic drugs and after finding no discernible evidence of any permanent deleterious effects, either mental or physical, Dr. Al-

lentuck considered the question of the possible therapeutic value of these substances. The potential availability of pure synthetics of standard potency invites such a study, for hitherto merely hemp extracts were accessible, the clinical activity of which must be determined for each batch of extracted material. Since the outstanding manifestation of the marihuana action is the euphoria which makes its user feel "high," consideration was given to its possible employment as a drug for individuals in various stages of mental depression as cyclothymics, involutionals, reactives, or those with organic conditions in which dysphoria is a dominant factor. The invariable characteristic of the drugs to stimulate the appetite, suggests they might be applicable in psychoneurosis in which a lack of desire for food exists. Many subjects show an alcohol-like picture of intoxication following the use of marihuana. The idea of using these drugs in the treatment of chronic alcoholic addiction was considered and preliminary experiments by Dr. Allentuck on private patients and colleagues were sufficiently encouraging to merit investigation on a larger scale and over a longer period of time.

The euphoria produced by marihuana is in many ways comparable to that achieved by the use of opium derivatives. This suggested the possibility of use in the treatment of opiate derivative addictions to eliminate or ameliorate the withdrawal symptoms commonly experienced during previously attempted so-called "cures." To clarify this question Dr. Allentuck selected a series of cases among drug addicts undergoing treatment. One group of thirteen received 15 mg. of tetrahydrocannabinol orally three times daily at 5:00 A.M., 2:00 P.M. and 10:00 P.M. and a sterile hypodermic injection; another group of fourteen received the same treatment without the sterile injection. Subjective and objective findings were recorded. In general the consensus of subjective opinions favored the new treatment as compared to previous cures and the established routine taken by some of these patients. They felt happier, had a better appetite and wanted to return to activity sooner. These results served as a basis for further study of fifty cases in which quantitative criteria were employed.

Two groups of twenty subjects were selected, one group receiving the tetrahydrocannabinol treatment up to a maximum of ten days and the others receiving none. Members of each group were observed throughout the day. Each morning they were interviewed and any complaints recorded on a chart. Thus an attempt was made to arrive

at a quantitative comparison of the withdrawal symptoms. It was found that the tetrahydrocannabinol treatment was useful in alleviation or elimination of withdrawal symptoms and in diminishing or eliminating the accompanying discomfort which follows cessation of narcotic indulgence. Any withdrawal symptoms under the tetrahydrocannabinol treatment were of a mild character and occurred within the first three or four days following which the patients began to feel much better. The chief complaints were restlessness, headache and dryness of the throat. They had an increased appetite and desire for food which diminished or eliminated such withdrawal symptoms as nausea, diarrhea and perspiration. They felt physically stronger and showed psychomotor activity. The feeling of euphoria produced by the tetrahydrocannabinol helped in rehabilitating the physical condition and in facilitating social reorientation. An outstanding result is a subjective feeling of relaxation. The sleep induced by the drug likewise contributes to the general improvement in the patients' health. These results are in contrast to those from the use of Magendie's solution which produces in the patients contentment for the first three or four days, after which signs of marked discomfort or withdrawal effects appear. The patients after this treatment, upon their discharge were shaky and generally in poor physical condition. These preliminary results with tetrahydrocannabinol justify a more exhaustive study of its possibilities as a means of relieving the withdrawal symptoms in narcotic addicts.

With this brief picture of the results of the coöperative program before you, I may conclude by adding a few remarks about what may be expected from a continuation of the investigations under way. In the chemical field, repeated attempts to synthesize a tetrahydrocannabinol with a double bond in the γ, δ-position have failed. Just recently, however, a new approach has appeared and the results have progressed to the point where I am convinced it is merely a matter of time before the goal is reached. The physiological reaction of this product will allow a conclusion in regard to the relative importance of the position of the double bond in the alicyclic ring and of the optical activity in the tetrahydrocannabinol molecule. Other synthetic molecules of a similar character, which are soluble in aqueous acids or bases and, therefore, perhaps suitable for intravenous injection, are being prepared. It is hoped also to clarify the significant groups and their orientation which induce marihuana activity. Thorough investigation of the constituents

in red oil is necessary to complete the understanding of hemp extracts.

In pharmacology, there is still much to be done in coöperation with the chemist to elucidate in more detail relationship between activity and molecular structure. With pure chemical substances of marihuana activity, it will now be possible to determine experimentally what actions are exerted upon body functions other than those which have hitherto attracted attention. The relationship between the mechanism of ataxia action in the dog and the psychic action in man should be clarified. It has not yet been established that the structural differences between the various marihuana-active substances do not result in a relative prevalence toward ataxia effectiveness by some, psychic effectiveness by others.

In the clinical field, the practical application of these substances must be awaited with the usual necessary patience. The initial experiments of Dr. Allentuck make it appear likely that some use of this interesting drug or its synthetic equivalents will be discovered.

In all phases of this work just completed, the groundwork has been laid so that a wider interest should ensue, and significant contributions may be anticipated in the chemistry, pharmacology and clinical aspects of this class of substances.

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LYMPHATIC PARTICIPATION IN CUTANEOUS PHENOMENA

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From the Laboratories of the Rockefeller Institute for Medical Research

Harvey Lecture, April 16, 1942

CIRCULATING blood does not come into actual contact with the cells which it nourishes. Instead fluids escape from the blood vessels and, after passing among the cells, return to the blood in part directly, in part after entering a vast system of collecting channels, the lymphatic system. How the fluid moves through the tissues or enters the lymphatics no one knows, but once within these vessels, the lymph, as it is now called, is sieved through the lymph nodes before it is poured back into the blood.

Far too long have the lymphatics been looked upon as a system of passive channels so constituted that fluid from the tissues merely seeps into them and drains away.

It is my purpose to present a wholly different conception: to draw your attention to some active functions of the lymphatic vessels of human and of animal skin. We shall not discuss the physiology of the lymphatic system as a whole, for our chief effort will be spent on the exposition of certain facts recently acquired and not generally appreciated, which show that cutaneous lymphatics in sickness and in health are most active not only in the everyday affairs of the skin but in the processes of immunity and in the defense of the body against infection.

The functions of the lymphatic system have remained largely unknown. Two influences have contributed to our state of ignorance, a lack of suitable methods of study, for the smaller vessels collecting the lymph are so thin-walled as to be invisible during life when examined by the usual techniques, and a lack of interest. The lymphatics were seen by the ancients, but were promptly forgotten and largely through indifference were ignored for nearly two thousand years. Then, in 1622, Aselli made his dramatic rediscovery of the lymphatics.[1] He had just

commenced a vivisection upon a well-fed dog before a small and select group of distinguished citizens of Pavia. He had opened the abdomen, gently put aside the intestines, and there in the mesentery to his amazement he beheld ramifying vessels filled with white fluid—the lymphatics distended with chyle. He described his findings in this way:[2]

. . . I suddenly beheld a great number of cords as it were, exceedingly thin and beautifully white, scattered over the whole of the mesentery . . . and starting from almost innumerable beginnings. . . . Wherefore struck by the novelty of the thing, I stood for some time silent while there came into my mind the various disputes, rich in personal quarrels no less than in words, taking place among anatomists. . . . When I gathered my wits together for the sake of the experiment, having laid hold of a very sharp scalpel, I pricked one of these cords . . . I had hardly touched it, when I saw a white liquid like milk or cream forthwith gush out. Seeing this, I could hardly restrain my delight, and turning to those who were standing by . . . more particularly to Senator Septalius, who was both a member of the great College of the Order of Physicians and, while I am writing this, the Medical Officer of Health, "Eureka" I exclaimed with Archimedes, and at the same time invited them to the interesting spectacle of such an unusual phenomenon. . . .

In the two hundred years following Aselli's discovery the early anatomists learned much about the abdominal lymphatics but only a little about the small lymphatics elsewhere. As late as the fourth decade of the 19th century it was generally believed that fluid was carried from the blood vessels to lymphatics by tiny vessels, the "vasa serosa." Belief in such connections was abandoned following Schwann's discovery of the cell, and Virchow[3] then suggested that the blood vessels and lymphatics communicated by hollow connective tissue cells. Next, von Recklinghausen[4] intimated that the smallest lymphatics opened into tissue spaces and that fluid entered through their open ends or through stomata much as rain water might be collected by drain pipes. But subsequent discoveries changed all this. First His[5] suggested that the lymphatics were closed vessels, a suggestion later to be proven true by the notable work of MacCallum[6] and of Florence Sabin[7] who, in 1915, reviewed some of her findings on this subject in a Harvey Lecture.[8] With this suggestion of His the mechanism of the formation of lymph became a mystery, and a fascinating one, yet to be solved. It was then that lymphatic physiology really began, for speculation and experiment

soon led to the two schools of Ludwig and Heidenhain, the one believing that lymph was formed by filtration, the other that it was secreted into the lymphatic capillaries by the cells of the wall. Lymphatic physiology made its greatest forward step under the influence of Starling[9, 10] and other English physiologists working in his time.[11, 12] Their work can be described briefly as the study of lymph itself, its constituents and its chemistry as bearing on its mechanism of formation, a subject that has been actively extended by Weech and his coworkers[13] and by Drinker and his group in Boston[14, 15] whose work has already been presented before this Society.[16]

Until very recently physiologists have contented themselves with collections of lymph from the larger lymphatics and with observations on the changes in the fluid under various conditions. As a result we know much more about what lymph is chemically than we do of how it forms or under what conditions it flows rapidly or slowly or transports substances from one part of the body to another. One technical difficulty has been that when a lymphatic is cannulated the contents are no longer subjected to the hydrostatic conditions present in the intact channel and the resulting flow from a cannula may be altogether different from that which would have occurred if the lymphatic had not been opened. There are other difficulties which are more considerable. Lymph is formed at the periphery and not in the larger vessels, and the formation of lymph can be understood only after more study of the minute vessels. But the ultimate lymphatics are too small to cannulate and, unlike the blood capillaries, they are invisible.

A few years ago Dr. Stephen Hudack and I utilized various vital dyes as an aid in observing the capillary blood circulation in the ears of white mice.[17] It was noted that when extremely superficial intradermal injections of minute amounts of solutions of these dyes were made at the tip of the ear, some of the coloring matter passed into lymphatic capillaries that had been torn or ruptured by the needle. As the dyes employed had no local tissue affinities they were carried along in the lymphatics with the result that the vessels were rendered visible from the tip to the base of the ear. One could see the smallest peripheral lymphatics as they passed through living tissue that was itself untouched and unharmed, and it became possible to test for the first time the reactions of these channels in health and disease. The ear of the mouse is ideal for such studies, for the anesthetized animal can be placed in

plasteline molds, with the ears spread upon porcelain plaques which serve as reflectors, rendering the most minute vessels visible under the microscope.

Activities of Lymphatics in the Ear of the Living Mouse

Fig. 1 shows the ear of a living mouse prepared in the way which has proved best for observational purposes. To render the lymphatics visible in this and in all the experiments in human skin as well as in the skin of animals, a minute puncture wound through the corium was made, under a binocular microscope, with a dissecting needle ground as fine as possible. The needle was then pushed for 2 or 3 mm. parallel to the surface of the skin and just under the epidermis. Into the tunnel so formed was thrust a gauge 30 platinum-iridium needle attached to a syringe, and a minute amount of dye, about 1/200 of a cc., was expelled with the least possible pressure. The dye promptly entered the lymphatic capillaries which had been torn or ruptured by the dissecting needle and within a few minutes extended along them to the base of the ear. One can see in Fig. 1 that the channels are typical lymphatics as the histologist knows them, irregular anastomosing channels with bulbous dilatations at the valves. If the colored contents of such vessels are pressed toward the periphery with a microspatula, no flow backwards will take place through the valves but instead the channels will rupture as a rule. Changes in the state of the tissues have much to do with the diameter of the lymphatics, which are at times wide, at times narrow. Spontaneous contraction or contractile response of the lymphatics of the ear to drugs or chemicals has not been encountered in our experience.

Evidence of Lymph Flow in the Resting Ear: Preparations like that shown in Fig. 1 have yielded evidence of lymph flow even in the resting ear. Active movement of dye can be seen to occur along the lymphatics of the ear but as it derived from the injected region it could not be taken as indicative of normal flow. However we have frequently observed, where two lymph channels joined, that dye-containing fluid was displaced and swept aside by another stream of clear lymph, itself unseen, deriving from areas remote from the region of injection, that is to say, from tissue untouched, wholly normal, and not edematous. The phenomenon could not have been due to transmission of pressure from the injected area, for recent work by micromethods[18] has shown that

interstitial pressure in the ear is not increased in such regions.

The Permeability of Lymphatic Capillaries: Previous knowledge of the permeability of the lymphatics has been inferential, being based upon comparisons of the blood and of the lymph from channels large enough for cannulation. But now the permeability of the walls of the smallest lymphatics can be tested directly, though it be in a direction opposite to that of normal flow. In a series of systematic studies[17, 19] vital dyes of graded diffusibility, in various concentrations and in different solutes, with and without protein, were introduced into the lymphatics and the rate of dye escape observed. Even the most indiffusible dye we could find, pontamine sky blue, escaped from the lymphatics. In recent work[18] the escape of this dye from lymphatics has been found to be no more rapid than that of T-1824, which is a dye so indiffusible that it is widely used for blood volume determinations.

Figs. 2 and 3 illustrate the characteristics of dye escape. Fig. 2 represents the ear of a living mouse with lymphatics containing 4 per cent pontamine sky blue dissolved in Tyrode's solution. In this photograph, taken 6 minutes after the introduction of the deep blue dye at the tip of the ear, escape has begun, as evidenced by the fuzzy appearance of the borders of the vessels. Fig. 3 shows the same ear 5 minutes later. Color has extended further from the lymphatics owing to the escape of dye and its secondary spread through the tissues. The passage of dye takes place everywhere along the channels.

As was to have been expected, highly diffusible dyes introduced into lymphatic capillaries escaped more rapidly than poorly diffusible ones. The addition of protein to the dye solution delayed its escape. Dye in high concentration escaped more rapidly than in low concentration. Dye dissolved in sodium chloride solution, isotonic with blood, escaped more rapidly from the lymphatics than did that dissolved in Locke's or Tyrode's solutions. Finely divided particulate matter, India ink or Hydrokollag[20] failed to escape at all during the periods of our experiments. In short, the lymphatic capillary wall was found to behave like a semi-permeable membrane.

Changes in Permeability of the Lymphatics: Sharp increases in the rate of dye escape followed mild stimulation of the skin, indicating that there had been alterations in the permeability of the vessels. To demonstrate these changes to the best advantage the most indiffusible dye obtainable, pontamine sky blue, in a 21.6 per cent aqueous solution

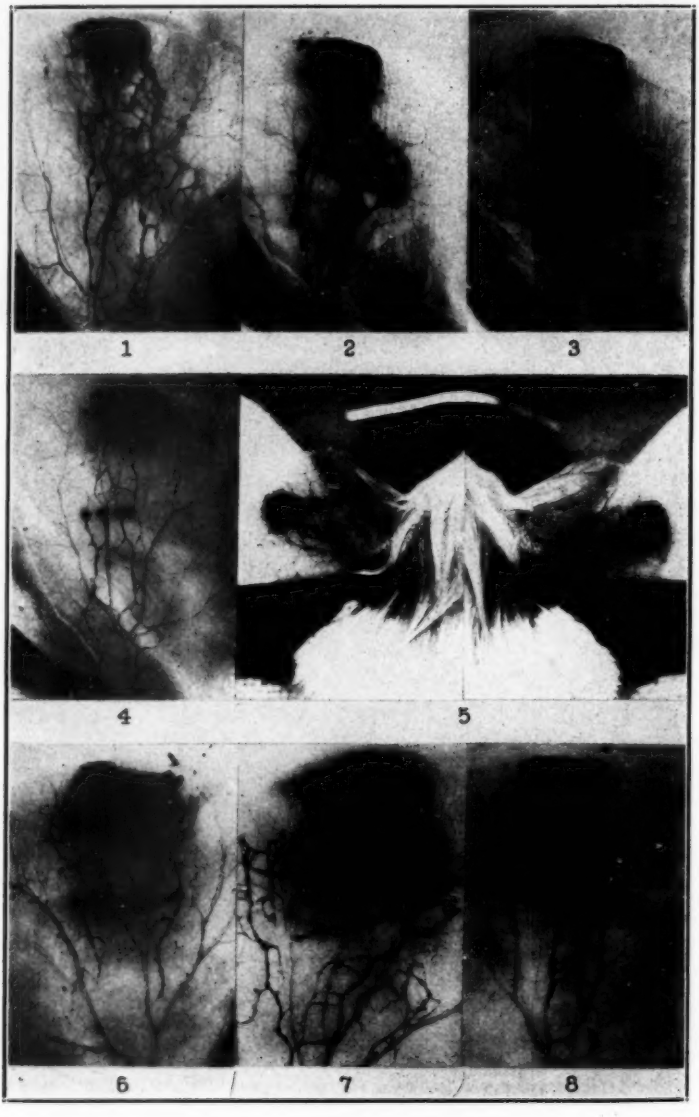
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Fig. 1—Ear of a living anesthetized mouse, photographed by reflected light, 16 minutes after the injection of the standard dye solution described in the text. The plexus of lymphatics, rendered sharply visible by their stained contents, lies in the corium. Other, deeper lymphatics can be dimly seen. But little dye has passed out from the lymphatics though these have been full of stained fluid since the injection was made, 16 minutes before. × 5.

Fig. 2—Ear of a living, anesthetized mouse with the lymphatics containing 4 per cent pontamine sky blue in Tyrode's solution. 6 minutes after injection of the dye, its escape into the tissues from the lymphatics is well under way. × 5.

Fig. 3—The same ear photographed 5 minutes later, that is to say, 11 minutes after injection. The color has extended further from the lymphatics, owing to progressive escape of the dye and its secondary spread in the interstitial spaces. × 5.

Fig. 4—Ear of a living anesthetized mouse photographed 6 minutes after the entry of standard dye solution into the lymphatic capillaries. 10 minutes prior to the injection the ear had been stroked transversely across the middle, as described in the text.

Sharply localized ecchymoses of dye appeared along the line of stroke, although this latter was so weak as not to elicit any reaction of the blood vessels. Under normal conditions no such escape occurs in ½ hour. × 5.

Fig. 5—The under surface of the right ear was warmed at 43.0-43.5° C. for 5 minutes. Both ears were then spread on plaques in the usual manner. 10 minutes after the heating, the standard dye solution was introduced into the skin and taken up by the lymphatics. The photograph was taken after a further interval of 10 minutes. It will be seen that dye has escaped profusely all along the lymphatic channels of the heated (right) ear, while only a little has occurred in the control (left) ear. × 2.

Fig. 6—Photograph of the ear of a living anesthetized mouse, injected at the ear margin with the standard dye solution described in the text, 5 hours after a transverse incision had been made in the skin of the upper surface. The dye entered the lymphatics of the injected area and gradually extended along them to escape from their severed ends, filling the wound with blue dye. × 5.

Fig. 7—The result of an intradermal injection of standard dye solution into the margin of a mouse ear, which had been incised 24 hours prior to the injection and photographed 5 minutes after it. The blood vessels and lymphatics had been cut through. Some of the dye reached and entered the incision but most of it was shunted around it as described in the text. The lymphatics were markedly permeable distal to the incision, as indicated by the abundant escape there, and much less so proximally. × 5.

Fig. 8—Lymphatics in the incised ear of a living mouse photographed 5 minutes after an injection of the standard dye solution. The incision was made the day before and is easily seen in the photograph. Three lymphatic channels, lying probably just beneath the incision, have conducted colored fluid past the region of injury into the tissue at the base of the ear. In doing so much dye has escaped into the incision. The increased dye escape distal to the incision, and the escape proximal thereto, are also well shown. × 5.

(isotonic with blood) was diluted to approximately 1 per cent with a mixture consisting of one part of mouse serum and three parts of Tyrode's solution. This yielded for all the experiments dye at a fixed concentration in a vehicle having the probable protein concentration of peripheral lymph. The character of this "standard dye solution," as we will term it, was such that when introduced into the lymphatics by the technique already described no escape of color could be perceived for 12 to 15 minutes, though shortly thereafter a barely perceptible coloration could be seen.

The readiness with which changes in the permeability of lymphatics follow the sort of stimuli that are encountered in everyday life is worthy of comment. For example, Fig. 4 illustrates the effect of a gentle stroke across the ear. In this experiment and many like it, the ear was stroked with the blunt, round handle of a probe, so gently that the skin was not broken or scratched: no reaction was elicited from the blood vessels nor were the latter ruptured. Ten minutes later the standard dye solution was injected at the ear tip and passed, in 2 or 3 minutes, through the lymphatic channels to the ear's base. In another 6 minutes the photograph was taken. The sharply localized ecchymoses of dye, like beads of a rosary, stand out in the line of the stroke. No dye has escaped elsewhere in the ear.

After such a disturbance of the lymphatics the state of increased permeability is maintained for 2½ hours. Very large molecules introduced into the lymphatics in such an experiment as when a hemoglobin solution is injected escape rapidly but particulate matter, India ink or finely divided graphite (Hydrokollag),[20] fails to escape. It is plain from this that the physiological barrier of the walls has been broken down temporarily, but not so their anatomical continuity.

Heat Increases the Permeability of Lymphatics: An extraordinary increase of lymphatic permeability results from the action of heat. The ears of anesthetized mice were allowed to rest on the outer surface of a hollow glass bulb shaped to fit the curvature of the ear. Water at any desired temperature could be made to flow through the bulb. Fig. 5 shows the result of such an experiment. The right ear was subjected to temperatures of 43 to 43.5° C. as it lay on the water chamber for 5 minutes. Ten minutes later both ears were injected at the margins, and they were photographed after another 10 minutes. In the unheated control ear almost no dye escaped, whereas the increased permeability of

the lymphatics of the warmed ear is obvious. Exposure to mild sunlight for half an hour brought about an increased dye escape from the lymphatics, while control experiments showed that in ears brought to the same temperature but kept shaded there was no such effect.

Mild irritation by chemical means produced the same phenomena. For example, a single application of xylol to the skin increased lymphatic permeability enormously. Greater degrees of disturbance had proportional results.

The experiments outlined so far carry certain implications. Influences which come within the realm of the normal—sunlight, slight warmth, a stroke which does not break the skin—these greatly but transiently increase lymphatic permeability. Such changes obviously have a meaning for local conditions. Fluid exchange between the blood and tissues is known to be altered by vasodilatation and contraction, alterations in the systemic blood pressure, and so forth. The lymphatics constitute a more passive system, yet much of their usefulness under this or that condition must depend upon the state of permeability of their walls. None of the injuries we have used so far to alter the permeability of the lymphatic wall breaks down the barrier so completely as to permit the immediate escape of particulate matter; yet insofar as the lymphatic is rendered more permeable to fluids by this or that influence, it ceases to be a walled off channel. We have shown that slight stimuli render the lymphatic wall so permeable that even hemoglobin passes it readily. What is true for the huge hemoglobin molecules cannot but hold for those of the plasma proteins.

Lymphatic Participation in the Repair of Incisions: The part played by the lymphatics in the healing of wounds and in the repair of connective tissue injuries has been largely unknown. Descriptions of the processes yield so little mention of lymphatics that one may well ask, do they share in these phenomena at all? To study their relation to the healing of wounds, incisions about 1 cm. long were made in the skin of mouse ears midway between the tip and the base. Some of the incisions were extremely superficial, some deep, the depth being controlled by observations with the binocular microscope. At varying intervals after making the incisions the standard dye solution was injected at the tips of the ears.

First of all it was found that the behavior of the lymphatics severed by incision differed greatly from that of the blood vessels.

Figure 6 shows the results of a typical experiment. Five hours after making an incision deep enough to sever superficial lymphatics and blood vessels dye was introduced into the lymphatics at the tip of the ear. It passed along the channels and escaped at their severed ends into the incision itself, although at this time constriction and spasm of the blood vessels prevented all bleeding. The picture, taken only 5 minutes after the injection, shows that the lymphatics had not only failed to close during the interval of 5 hours but that the channels distal to the incision were far more permeable than normal, as evidenced by dye escape. Intravenous injections of dye in other experiments showed that the blood vessels about the incisions were also more permeable than normal.

When a drop of dye solution was placed in a superficial cut the lymphatics drained away colored fluid to the base of the ear, showing that the channels were still open. These phenomena were not invariable and whether or not they took place seemed to depend upon how dense a fibrin clot had developed in the wound, hindering access to the lymphatics. The fact that severed lymphatic vessels often remain open for considerable periods of time, unlike the blood vessels, and lead away fluids from wounds readily explains the frequency of infection by way of the lymphatics.

In a few experiments, instead of injecting the incised ears, a tiny crystal of dye was pushed, under the guidance of the microscope, into a minute intradermal puncture wound at the periphery of the ear. Within 15 to 20 minutes colored fluid could be seen passing from the severed lymphatic capillaries into the incision. This occurred of course without application of pressure.

In the experiments in which dye was introduced into the tissues, by hand and through a needle, "the least possible pressure" was employed. Very recent work, which cannot be detailed here, shows that the pressure of such an injection is about 6 to 12 cm. of water. The pressure within lymphatic capillaries of a motionless mouse ear was found by micromethods to be 0.7 ± 0.3 cm. of water, but pressures of only 2 to 4 cm. of water will cause a flow of dye into incisions even several hours after they have been made. From a practical point of view, then, the slightest touch on the skin in dressing a mouse wound will produce results like those seen in the photographs. There is good reason to suppose that the same will be found to hold true for man.

In tests made 24 hours after incising the skin, the dye injected at the ear's tip was mostly carried to within about 2 mm. of the incision and then shunted around it. Only a little reached the wound itself, arriving there from the channels skirting either end. Fig. 7 shows the results of such a test. The photograph was taken 5 minutes after an intradermal injection into an ear incised 24 hours before. Both blood vessels and lymphatics had been cut through. The finding portrayed was that generally obtained but in a few instances dye escaped into the wound, and furthermore, entered directly into lymphatics on the other side of it. Intralymphatic pressures of 30 to 40 cm. of water, brought to bear by micromethods,[18] invariably gave such a result. Fig. 8 shows a photograph of an instance. That lymphatic channels distal and proximal to the incision were abnormally permeable to the dye is obvious in both Figs. 7 and 8.

Forty-eight hours after making an incision, dye intradermally injected by hand with the least possible pressure at the ear's tip regularly failed to enter the incised area and was shunted around it. Intralymphatic pressures of 60 to 80 cm. of water were required to force dye into the incision, such pressure probably dislodging fibrinous plugs in the lymphatics. The permeability of the lymphatics proximal to the wounds seemed to have returned to normal, as judged both by the rate of dye escape, and by the speed at which the channels filled with dye became decolorized as new fluid washed it out. On the other hand, intravenous injections of dye 48 hours after making the incisions showed that the blood vessels all about the incised area were still far more permeable than normal. More will be said of this below, after discussing the changes in the lymphatics about burns.

New Formation of Minute Lymphatics in Areas of Repair: Clear evidence was obtained of the new formation of lymphatics in ears studied 7 to 10 days after incision of the skin. Lymphatics at the periphery of the ear, injected in the usual way at this time, led dye solution or India ink into a wealth of small channels in and about the cuts. Frequently the injected fluids passed, in reconstituted channels, directly through the incised areas. Fig. 9 shows the result of an injection of India ink at the margin of the ear 9 days after making an incision deep enough to sever lymphatics and superficial blood vessels. On the left side of the incision several lymphatics are seen carrying ink either directly through the healing incision or just beneath it. The India ink was used for

purposes of photography, to show the outline of the lymphatics unobscured by the escape of dye. The line of the incision lies between the two arrows on the photograph.

The Participation of Lymphatic Capillaries in the Reaction about Burned Areas: Small, sharply localized, standardized first, second and third degree burns of the ears of mice were made by applying to the skin thin-walled glass water chambers through which water circulated at any desired temperature. Local injections of standard dye solution into the lymphatic capillaries of the uninjured tissue at the ear tip resulted in the entrance of the colored fluid into channels which passed directly through the burned areas, or under them, and emerged again in normal tissue at the ear base. The application of heat of 55° C. for 45 seconds to 1 minute produced a mild first degree burn. Fig. 10 shows the result of an experiment in which dye entered the lymphatics 6 minutes after burning the skin in this manner. Only 2 minutes later the picture (Fig. 10) was taken. The fuzzy escape of dye along the lymph channels in this very brief period outlines the area of the burn, indicating a great increase in permeability of the lymphatics. After another 2 minutes a second photograph (Fig. 11) was taken. The speed of dye escape in this short interval is obvious from the spread of color in the tissues. It is to be noted that dye escaped from the channels only in or near the mild burn.

Occasionally dye introduced into the lymphatics at the margin of the ear passed directly through small second or third degree burns if the injection were made not too long after the skin had been injured. The great increase in the permeability of the walls of lymphatics in or near such burns is shown in Fig. 12. In the experiment from which this photograph was taken a punctate third degree burn, the clear area in the picture, was made 3 hours before the injection of dye by applying to the skin for 45 seconds a small water chamber heated to 60° C. The photograph, taken only 3 minutes after the injection, shows the extent to which the lymphatics at the edge of the burn and one traversing it have poured their contents into the injury. In this experiment we employed, as in the previous one, the standard dye solution which escapes visibly in the normal ear only after 12 to 15 minutes.

For 24 to 48 hours the sharply localized second and third degree burns, made in the manner described, remained as ischemic patches on the ears, surrounded by regions of hyperemia and edema. Twenty-four

hours after the formation of a mild burn, dye injected into the lymphatics at the ear margin was mostly carried around the region of injury through extremely permeable channels. The lymphatics leading directly to the burned area seemed to terminate in blunt ends, closed perhaps by heat coagulations or fibrinous clots. Occasionally lymphatics transported dye directly through the burn, and when this happened the color escaped so rapidly that one might doubt the existence of lymphatic walls were it not that India ink or other particulate matter similarly injected did not escape.

By the 2nd day the lymphatics which filled most readily, that is those which skirted the burn, began to show normal permeability again. Dye passing along them was carried away instead of escaping from the walls. The finding was of some interest because at this time the blood vessels about the burn were still far more permeable than normal. Fig. 13 illustrates this fact. It shows a ring of intense color about a burn 2 days old due to the escape of dye from the blood vessels 4 minutes after an intravenous injection. Dye had escaped from the highly permeable blood vessels about the burn and not from vessels elsewhere in the ear. At this time the lymphatics had begun to regain their normal permeability.

In the later stages of the repair of burns the lymphatics showed notably active proliferation within the recovering tissue. An example is shown in Fig. 14. In the experiment providing this photograph perforation had resulted from a localized burn of the ear made 9 days before. Twig-like lymphatic capillaries are visible, growing into the ring of newly formed tissue which is closing the perforation, while the abnormally rich plexus of vessels proximal to the injury signifies the fact that new lymphatic capillaries are not only growing into the new tissue but are all about it.

The observation that lymphatics regenerate is, of course, not new. The phenomenon has been described by Lee[21] and by Colin[22] for the thoracic duct, by Reichert[23] for the lymphatics of the limbs, by Clark and Clark[24, 25] for those of the rabbit's ear, and by others. For our purposes the point of interest lies chiefly in the enormous number of apparently new vessels which appear in the recovering tissue. This observation, which indicates great activity of the lymphatic system in the processes of healing, has been confirmed by Pullinger and Florey,[26] whose work has been cited by Drinker and Yoffey.[14]

The implications of these findings are not inconsiderable. It is well

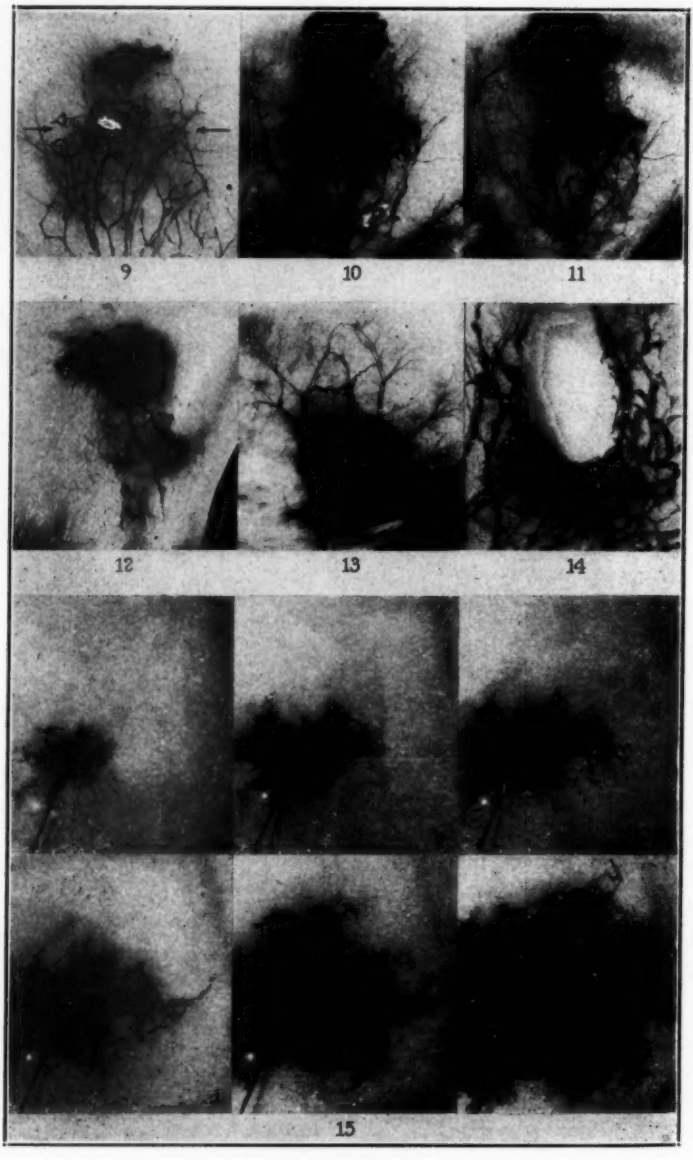
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Fig. 9—Demonstration with India ink of the lymphatic plexus about a healing wound. 9 days previously the skin had been incised. In the photograph the incision lies in the line between the two arrows. On the left side of the incision several lymphatics are seen carrying ink either directly through the healing incision or just beneath it. × 4.

Fig. 10—This photograph shows the escape of the standard dye solution into a burned region. 8 minutes prior to taking the photograph a chamber containing water at a temperature of 55° C. was brought into contact, for 40 seconds, with an area midway between the tip and base of the ear. 6 minutes later the dye injection was made near the tip, and 2 minutes later the photograph was taken. The area of dark, fuzzy escape of dye along the lymph channels coincides with the area of the burn. Elsewhere no dye has passed out. × 4½.

Fig. 11—The same preparation 2 minutes later, that is to say, 4 minutes after the injection. In unharmed ears standard dye solution does not begin to escape from the lymph channels for 12-15 minutes. × 4½.

Fig. 12—A photograph taken 3 minutes after a local injection of standard dye solution at the margin of the ear. 3 hours prior thereto a punctate burn midway between the tip and the base of the ear had been made by heat at 60° C. applied for 45 seconds, as described in the text. Dye passed directly through the burn in one lymphatic and was carried close to it in other channels. The rapid dye escape from the lymphatics in and close to the burned region is shown. × 4.

Fig. 13—A photograph of the ear of a living anesthetized mouse 2 days after a punctate burn had been induced on its upper surface. 4 minutes prior to the photographic exposure the animal received intravenously 0.05 cc. of aqueous isotonic pontamine sky blue solution (21.6 per cent).

The increased permeability of the smaller blood vessels is evidenced by a ring of intense color about the burn, while elsewhere in the ear very little dye has escaped. At the center of the burned area there is some slight diffuse staining. × 5.

Fig. 14—The ear of an anesthetized mouse injected with a suspension of dialyzed India ink in 5 per cent gelatin solution 9 days after a stigmatic burn. The burn had caused a complete perforation of the ear which at the time of the injection was gradually being closed by granulation tissue. Several very small twig-like lymphatics can be seen in the new-formed tissue and about the healing burn there is an abnormally rich plexus of lymphatics, many of which are very small. × 12.

Fig. 15—Successive stages in the distribution of dye during an intradermal injection into the skin of the volar surface of the arm of a living human being. The photographs were selected from a moving picture film and were taken 12, 21, 32, 45, and 65 seconds, and 3 minutes, respectively, after the beginning of the injection which lasted 68 seconds. Note that the injecting needle is seen in the first five photographs but not in the sixth. × 2½.

known from the work of many authors that blood vessels in and about regions of injury, mild or severe, are more permeable than normal. Menkin, who has recently reviewed the subject,[27] has produced evidence which indicates that substances escaping from the abnormally permeable blood vessels into regions of severe injury and inflammation are fixed there, and furthermore that the lymphatics leading away from these regions may be partially or completely obstructed by fibrin deposits. Such a state of affairs leads to the partial isolation of severely injured regions from the remainder of the body. Our studies show that in and about mild burns, as in and about mild injuries, the permeability of the lymphatics is enormously increased without loss of the anatomical continuity of their walls. The profound alterations in permeability speak for active participation of the lymph system in the changed processes of fluid exchange. Like the blood vessels, the lymphatics respond to injury first by pouring their contents into the region involved, not only into the injured area itself but all about it. As our observations show, the lymphatics regain their normal permeability before the blood vessels do, and through the lymphatics resorption from injured areas seems first to begin. One may suppose that through them the noxious products resulting from injury are carried away and sieved through lymph glands before reaching the body at large.

Studies of the Lymphatics of Living Human Skin

The physiology of human skin has been written as though blood vessels, nerves, and interstitial fluid alone were involved in its activities. The lymphatics have been almost ignored. To learn what goes on in them the techniques just described were applied to human skin and by their use the lymphatics of living men were rendered visible for the first time.

The injections into human skin were made as already described for the mouse ear save that a more diffusible dye, patent blue V,[28] was employed to avoid enduring discoloration of the skin. The six photographs of Fig. 15 illustrate the results of an intradermal dye injection on the volar surface of the forearm of a normal subject. The photographs presented were taken at intervals of approximately 15 seconds after the beginning of the injection which lasted slightly over a minute. The channels rendered visible by the dye lie in the subpapillary layer of the corium and the lymphatic plexus there is far richer than had previously

been supposed. In the past gelatin injecting masses have been usually employed to study them, thicker mixtures which enter only a small fraction of the channels present in the skin. We have found too, as the photographs show, abundant anastomoses, which deserve emphasis because of a further finding, to be described below, that every wound through the human corium tears lymphatics open and allows foreign material to enter them directly. Indeed the lymphatic plexus is so close-meshed that one cannot make an intradermal injection without injecting the lymphatics.

Fig. 16 shows the results of two intradermal injections of dye in a living arm. Pigment, pale because diluted with lymph, is beginning to drain from the injection site into subcutaneous lymphatics and can be seen under the skin as dimly visible colored streamers ascending the arm. The invariable occurrence of such streamers following intradermal injections of dye has suggested that dyes might be used to study changes in cutaneous lymph flow. To test the point they were employed as will now be detailed.

For injections like those photographed in Figs. 15 and 16 nearly 0.1 cc. of an 11 per cent solution of the dye, patent blue V, was employed.[28] Though the bulk of fluid introduced was so small, dye drained rapidly away into the deeper subcutaneous trunks. In the experiments to be discussed below far less dye solution was injected, only 0.01 to 0.02 cc. for each test. Further, the concentration of the dye solution was reduced to 1 per cent, which yielded just enough color to be visible in lymphatics under the skin.[29,30] The decrease in concentration and volume of material injected reduced the amount of pigment to about 1/50 of that previously employed. The resulting streamers formed slowly, requiring about 20 minutes to become 10 to 15 cm. in length. As a result variations in their length and intensity could easily be distinguished.

In Fig. 17 (*a-e*) natural size photographs of the results of a minute injection of the sort mentioned have been reproduced. They were taken at intervals of 30 and 45 seconds, 1, 2, and 3 minutes, respectively, after the dye first began to enter the lymphatics. The injection in this instance required only 52 seconds. All the injections to be considered below required less than 1 minute. A greatly reduced photograph of the arm (Fig. 17*f*) shows a characteristic streamer which developed in the ensuing 20 minutes.

It is to be stressed that following a minute injection of this sort the

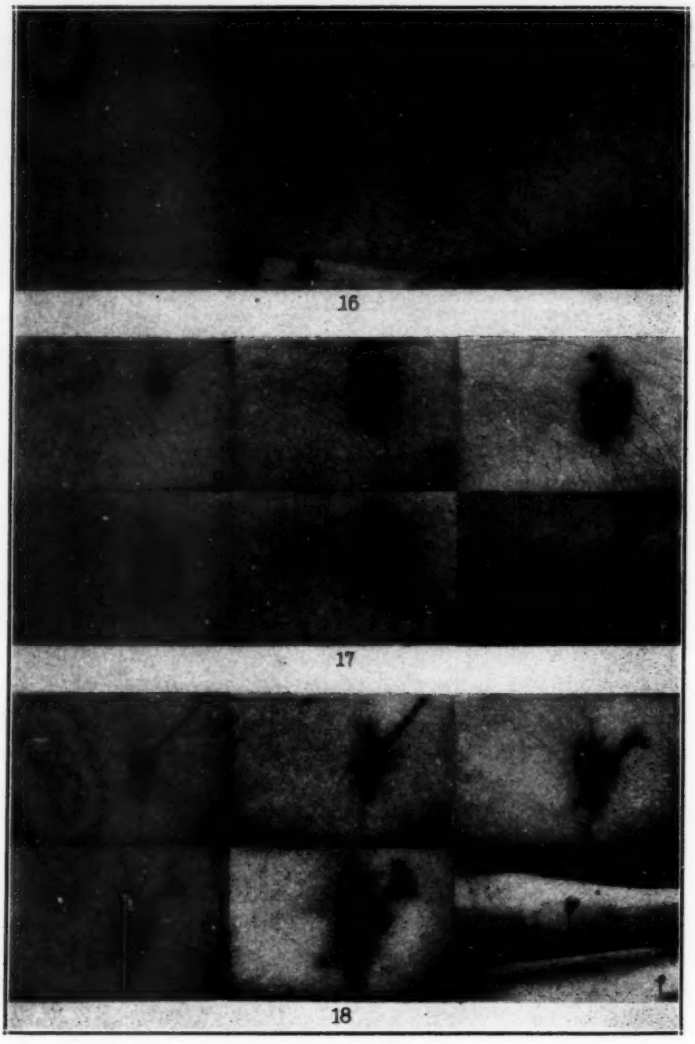


Fig. 16—The aftermath of two intradermal injections of a vital dye, patent blue V, into the skin of the volar surface of the arm. Dye, pale because diluted with lymph, is draining up the arm in subcutaneous lymphatics. × ⅓.

Fig. 17 *a-e*—Natural size photographs of the distribution of dye on intradermal injection into the skin of the volar surface of the forearm. The photographs were taken 30 and 45 seconds, and 1, 2, 3, and 20 minutes after beginning the injection. Fig. 17 *f* is reduced to 1/7 natural size and shows a colored streamer extending up the arm from the injected area.

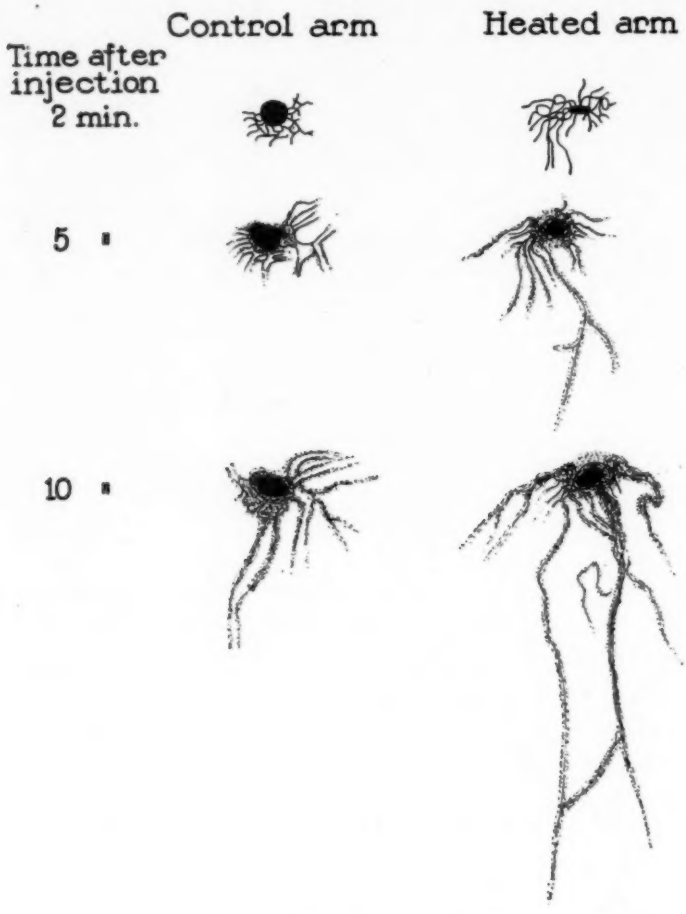
Fig. 18—Results of an intradermal injection of dye into the skin of the volar surface of an arm during a period of venous obstruction, caused as described in the text. The injection was made after pressure had endured for 20 minutes. Fig. 18 *a-e* was taken at the same time intervals as Fig. 17 *a-e*. Fig. 18 *f*, however, shows only part of an intense blue streamer which formed in 2 minutes following the release of the venous obstruction. × 1/7.

development of a streamer is a very different phenomenon from that obtained when larger amounts of dye are forcibly injected into skin to obtain anatomical preparations of the lymphatics. Under the latter circumstances undiluted dye is actually forced into the lymphatics under great pressure and is not transported by flow along them. In our experiments minute amounts of dye solutions, isotonic with blood, are injected with the least possible pressure into the tissues. From the injected area the solution, diluted by tissue fluids, extends very slowly into superficial lymphatics, there to be still more diluted. Slowly, and only after some minutes, it reaches the lymphatic trunks as dye at the site of the injection is further diluted by tissue fluid, to become lymph. To be sure, some pressure is unavoidably employed in making an injection but, as already mentioned, it is slight indeed, varying from 6 to 12 cm. of water. Recent work has shown that the pressure in the bleb of injected dye is only 4 to 8 cm. of water and falls rapidly to become equal within 4 minutes to the usual interstitial pressure of less than 2.0 cm. of water.

Changes in Lymph Flow Reflected by Changes in the Length and Intensity of the Colored Streamers

The Effects of Agents Known to Stimulate or Retard Lymph Flow: To test whether or not changes in lymph flow in human skin find reflection in changes in the length and intensity of the streamers, use was made of the common knowledge that lymph flow is increased by applications of heat,[9,15] by massage,[13,15] by activity,[31,32] or by hyperemia,[33] and that it is diminished in limbs that are at rest.[9,13,14]

Constant amounts of standard dye solution were injected into corresponding skin areas of the arms and legs of normal subjects whose limbs were then subjected to one or another of the conditions just mentioned. Tracings of the streamers as they developed were made on strips of celluloid held over the skin. Text-fig. A shows a series of tracings of streamers which developed from two similar injections made at approximately the same time on the volar surface of the resting forearms of a normal subject. One arm remained at room temperature while the other was submerged in warm water at 46-47° C. Both arms were motionless, at the same level below the apex beat of the heart. Both injections were made with the same amount of dye solution, at the same pressure, and the conditions about the injection site were similar. Heat, which is



Text-Fig. A.—The effect of warmth to increase lymph flow. Tracings of two similar intradermal injections of dye in the forearms of the same subject, 2, 5 and 10 minutes after injecting. Dye escape from the lymphatics is indicated by the stippling. Column 1 shows the result in the normal resting arm, column 2 in the arm resting in warm water at 46.0-47.0° C. In the warmed arm streamers developed more rapidly. Natural size.

known to increase lymph flow, increased both the intensity and the length of the streamers. Ten experiments all gave similar results.

When an arm injected in the way described was used to pummel a punching bag, intensely colored streamers developed and reached the shoulder in a minute or two. Massage also brought about the rapid for-

mation of intense streamers, by an actual squeezing of the dye along the channels. Passive movement yielded less pronounced effects than active movement but nevertheless a definite increase in the length and intensity of the streamers was seen. In all our experiments the streamers were shortest and least colored in resting limbs.

The effects of posture were striking. In an arm intradermally injected with dye and held vertically downward at rest, streamer formation was absent; whereas when the same arm was raised vertically above the head and then injected, it was rapid. Streamer formation was absent in the injected lower legs of normal subjects seated quietly with the feet resting on the floor, but conspicuous if, a few minutes later, the injected leg was elevated and propped on a table while the subject remained seated. Streamer formation was still greater if a limb which had been hanging downward was raised and immediately injected.

The lack of streamer formation in the dependent limb is of much interest. It is well known that in the dependent limb fluid collects; the mechanism is charged as it were for lymph formation yet no streamer develops. If a lymphatic is cannulated under these circumstances, lymph flows from the cannula, for the back pressure from the vertical column of lymph above the point of cannulation no longer exists, as in the intact system, to prevent the movement of lymph.

It is to be noted that in the tests on human skin, so far described, the streamers showed great differences under different circumstances although the conditions of injection, that is, amount of dye, concentration, pressure, and the local edema at the site of injection, were the same. Procedures known to increase lymph flow caused an enhancement in the size, number and intensity of the colored streamers developing after injection of small amounts of dye in the skin. In resting limbs, in which presumably lymph flow was least, streamer formation was least.

The Effects of Some Conditions Having a Problematic Influence upon Lymph Flow: With these facts established, we studied next the changes in streamer formation brought about by conditions having an unknown influence upon lymph flow. No streamers ever appeared in the skin of the forearm or lower leg if pressure, even as little as 10 mm. of mercury, was applied to the upper arm or leg by a pneumatic cuff. By contrast the most rapid formation of streamers that we have ever seen occurred in motionless limbs during the reactive hyperemia that

follows release of obstruction to the circulation.

Fig. 18 (*a-e*) shows some photographs of the skin of a forearm injected with dye 20 minutes after bringing about venous obstruction by inflation of a Riva-Rocci cuff to a pressure of 90 mm. of mercury. The photographs were taken at the same time intervals as in Fig. 17. No streamers developed during a further 20 minute period of obstruction. The final photograph, Fig. 18*f*, shows only part of an intense blue streamer which formed in less than 2 minutes after release of the pressure cuff. The streamer at that time reached the shoulder. No streamer so long or intense was ever found at any time after a similar injection in a normal arm.

Fig. 19 *a* and *b* shows the result of an experiment in which several dye injections were made intradermally during a 40 minute period of total circulatory obstruction. During this period no streamers developed. The obstruction was then released and the streamers shown in Fig. 19 *a* appeared in 1½ minutes. Fig. 19 *b* shows them again after a further interval of 1½ minutes. The great increase in intensity is striking. The phenomenon occurs during the intense reactive hyperemia which invariably develops almost immediately after release of the obstruction.

Influence of Pulsation of Blood Vessels on the Movement of Lymph in Motionless Tissues

It is noteworthy that streamers representing the movement of colored fluid through the lymphatics can develop with such great rapidity in a motionless limb. How can one account for this? It is well known that massage, muscular movement and other mechanical factors increase lymph flow. But in the resting limb what mechanical forces are present? Can it be that the pulsation of blood vessels acts to further the flow? To test the point, we resorted, together with Robert Parsons,[34, 35] to experiments on the rabbit's ear.

In preliminary tests minute amounts of dye solutions were injected intradermally at the tips of the ears of many rabbits. Colored streamers appeared like those in human skin, reaching to the base of the motionless ear in about 15 minutes. In each experiment many lymphatic capillaries and small lymphatics, which contained color 10 to 15 minutes after the injection, became cleared a few minutes later. Often these channels lay far toward the base of the ear, too far away from the injected area to be subject to influence by any local edema there. Clearly lymph flowed

into those channels from regions of the ear that were untouched and, in sweeping out the colored fluid already there, gave evidence of lymph flow from areas of motionless tissue. When the ears were warmed to 45° C. they became hyperemic, and under these circumstances the blue streamers reached the base much faster than in the normal ear. Channels containing color a few minutes after the injection were cleared much more rapidly too. That is to say, hyperemia, which is known to increase lymph flow, hastened the rate of streamer movement and the clearance of channels in the rabbit's ear.

The ears of rabbits were next perfused with defibrinated rabbit's blood in such a way that a pulsation simulating the natural could be imparted to the fluid or withheld at will. In the absence of pulsation there was almost no movement of lymph, whereas when pulsation was present, lymph flow, as estimated by the streamer formation, was 15 to 20 times more rapid despite the fact that the "systolic" pressure in the pulsatile perfusions never exceeded the constant pressure of the non-pulsatile perfusions and the volume flow in the former was often but one-seventh that in the latter. Variations in the pressure and flow in these experiments demonstrated clearly that the mechanical effect of the pulse increased the movement of fluid both into and along the lymphatics.[34,35]

Other Mechanical Forces Favoring Lymph Flow in Motionless Tissues: Micromethods have been used to measure the intralymphatic capillary pressure in the skin of mice and rabbits, as also the pressure required to produce flow in the capillaries and interstitial pressure, often called "tissue pressure," outside the capillary wall. Under normal circumstances the intralymphatic capillary pressure is approximately 0.7 ± 0.3 cm. of water and the pressure required to produce flow is 0.3 to 0.5 cm. of water higher. The interstitial pressure outside the lymphatic capillary wall has never yet been found lower than the pressure required to produce lymph flow. Occasionally the two pressures have been found equal, but in nearly all instances the interstitial pressure has been higher by 0.5 to 1.5 cm. of water. In conditions of inflammation or suddenly forming edema the interstitial pressure may exceed by several centimeters of water the pressure required to produce lymph flow. As a result there exists usually a gradient of pressure tending to force fluid from the tissues into and along the lymphatic capillaries.

The experiments upon the ears of rabbits afforded us our first oppor-

tunity to determine whether streamer movement represents the actual movement of lymph in a lymphatic or whether the streamers are artifacts. The point is of much importance, for physiologists generally regard the lymph flow from motionless limbs as negligible. We have seen that colored streamers in resting human limbs extend 10 to 15 cm. in about 20 minutes. Under the circumstances of our experiments, there is an appreciable movement of colored substances introduced into the lymphatics of the skin of a resting limb and, as we have described, much more movement in a limb moved or subjected to changes in pressure. One may well ask: Do the streamers which develop in normal arms represent the movement of lymph taking place normally or is there an artificial movement caused by local edema at the site of injection?

A way was found to test the point by experiments upon the ears of rabbits. On numerous occasions lymph was collected from one of the large lymphatics at the base of the animal's ear. The flow of lymph in the motionless ear was found much greater than that reported by Henry,[36] who collected lymph in a similar manner. In our experiments, after collecting lymph for 1 to 2 hours, an intradermal injection of dye was made in the usual manner at the ear tip. Color appeared in the lymphatics at the tip as usual and passed to the channels at the base of the ear and into the cannula in approximately the same time that it does in normal ears. In most of the experiments, during this period the rate of flow of collected lymph did not change. In the remainder an increase of less than 10 per cent occurred.

In many cases the ear was warmed to 44° C. after lymph had been collected at the ear base for 1 to 2 hours. Dye reached the channels at the base of the ear and entered the cannula in about half the time required in the preceding experiments. The flow of lymph into the cannula increased greatly, the increase in flow being approximately proportional to the increase in the rate of movement of the streamers.

One can conclude that there is slight lymph flow from the skin of bodily regions that are at rest and that the injection of minute amounts of dye, 0.01 to 0.02 cc., as in our experiments, either does not affect the rate of flow or augments that already present by less than 10 per cent. Hence the rate of formation of streamers gives a close indication of the rate of lymph movement from uninjected tissues. In this connection attention should be called to the fact that the volume flow represented by a streamer moving 10 to 15 cm. may be very small because the skin

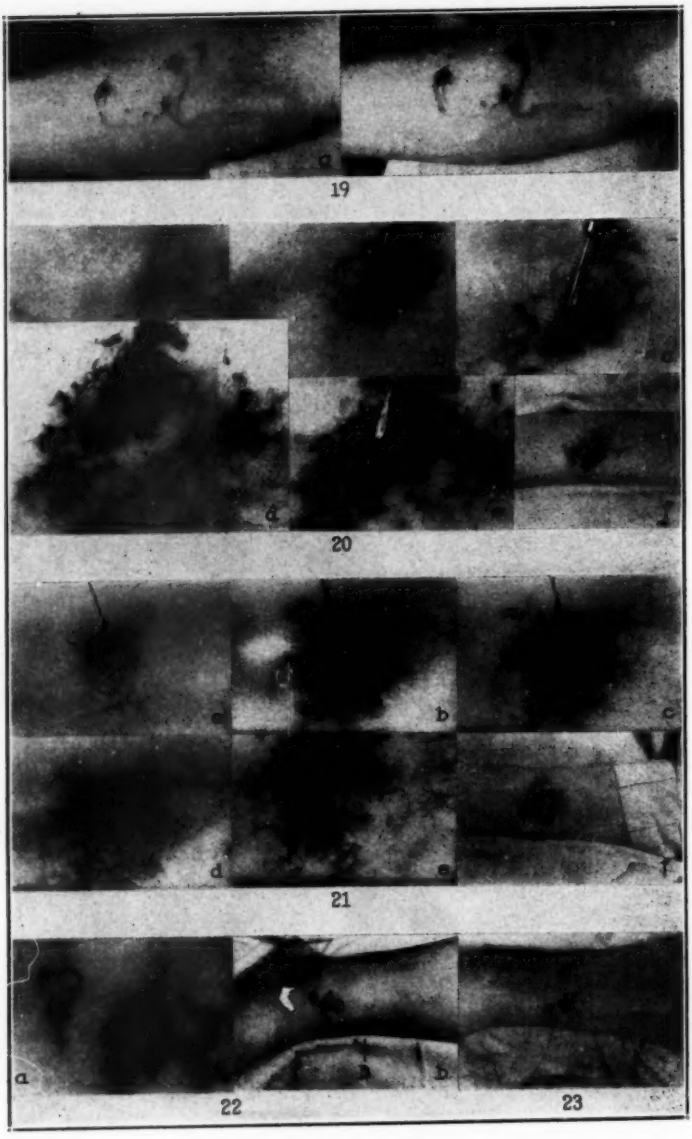
lymphatics, though broad, are flattened and ribbon-like. The point of chief interest to us in this relation is not that a certain volume of lymph in cubic millimeters or cubic centimeters flows through a given channel, but that substances entering a lymphatic through a scratch or puncture can be carried far in a short time.

Lymphatics and Lymph Flow in Edematous Skin of Patients With Cardiac and Renal Disease

In certain types of cardiac and renal disease fluid collects in the skin with result that edema makes its appearance. Do the lymphatics fail in their function of tissue drainage under these circumstances and add to the abnormality or do they assist in recovery? Since the dye injection technique clearly demonstrates known changes in lymph flow, it was used to answer this question. Tests like those made in normal skin were repeated in the skin of the legs of patients suffering from cardiac decompensation. All were elderly patients with no clinical signs of primary renal disease. Studies were made on each patient while edema increased, when it was stationary, and during resorption.

The dye showed the lymphatic capillaries of the skin to be widely dilated and the intercommunications between the channels extremely rich. The coloring matter when first introduced spread further along the lymphatics and escaped from them more readily than from those of normal skin. Yet no evidence of lymph flow was obtained in any of the tests, streamer formation being totally absent. At the periphery of the injected regions there was abrupt paling, obviously from dilution of the dye in the lymphatics by the copious lymph already present in the channels. Even in tense, distended skin of dropsical patients the lymphatics were widely open but they were full of stagnant lymph. We have reported the dilatation of lymphatics in the edematous ears of mice[19] and Pullinger and Florey[26] later described and pictured the phenomenon.

Fig. 20 (*a-f*) shows the appearance of lymphatic capillaries in the edematous leg of a cardiac patient while edema was on the increase. It is typical. The photographs were taken 10, 15, 25 seconds and approximately 1, 3, and 20 minutes, respectively, after beginning the injection. The last photograph, Fig. 20 *f*, shows the extent to which dye had spread in the lymphatics in 20 minutes. Yet there were no colored streamers. Several weeks later the state of affairs in the same patient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while rapidly losing his edema was similar. This fact is obvious from Fig. 21 (*a-f*), taken at approximately the same time intervals following injection as Fig. 20 (*a-f*). There were still no colored streamers, no evidence of lymph flow.

In many instances of long standing cardiac edema, there appeared during the course of the intradermal injection isolated "islands" of dye-containing superficial lymphatics several centimeters away from where the needle had entered. These "islands" were separated from the immediate area of staining by skin of normal hue (Figs. 22 (*a* and *b*) and 23) and were never seen under normal circumstances. It is plain that some of the injected pigment entered the deeper plexus and passing along this emerged again in the superficial plexus. The "islands" appeared below the site of injection as well as above it or at the side, as shown by the arrows "A" and "B" in Fig. 22 *b*.

No matter how much the lymphatic channels were dilated in cases of cardiac edema, we never observed the formation of colored streamers. There was no evidence of lymph flow, yet the fact that the lymphatics

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Fig. 19 *a-b*—Streamers developing 1½ and 3 minutes, respectively, following the release of total circulatory obstruction as described in the text. × 1/5.

Fig. 20—Spread of dye in the edematous skin of the ankle of a patient suffering from cardiac insufficiency. The natural size photographs were taken at 10, 15, and 25 seconds, respectively, after beginning the injection, which lasted only 37 seconds. Fig. 20 *d*, composed of parts of four overlapping photographs taken at 10 second intervals from ¾ of a minute to 1¼ minutes after beginning the injection, shows the size of the injected area. The lymphatic capillaries are seen to be widely dilated and dye escape from them has been rapid. Fig. 20 *f*, taken 20 minutes later, and reduced to 1/10 natural size, shows the large area then covered by the injection and the absence of deep streamers of dye.

Fig. 21—Results of a dye injection into the ankle of the same patient several weeks later when he was rapidly losing edema. The photographs were taken after intervals of 18, 25, 35, and 55 seconds, and 2 minutes 20 seconds and 20 minutes from the beginning of the injection, which lasted 50 seconds. Again no colored streamers can be seen. The magnifications are similar to those of Fig. 20. The central pale splotches in these and other photographs are high lights caused by dye solution that had escaped on the surface of the skin.

Fig. 22 *a-b*—Result of an intradermal dye injection into the edematous skin of a patient with cardiac edema. The photograph (natural size) was taken 3½ minutes after beginning the injection, which required 48 seconds. Fig. 22 *b* (1/10 natural size) was taken 20 minutes after beginning the injection. Islands of dye appeared as described in the text. No streamers were visible.

Fig. 23—The same phenomenon as in Fig. 22 *b* but as it appeared in a different patient ½ hour after injection. × 1/10.

were patent could readily be demonstrated. When a region stained as result of an intradermal injection of dye was massaged, colored streamers promptly appeared. If the skin of the lower leg of a patient with long standing edema was stroked from the injection site toward the periphery, a retrograde passage of dye took place along the superficial lymphatics. The phenomenon was never seen in normal man, nor did it occur in the patient a few days after the edema had been reduced by therapeutic measures. It was plainly indicative of a valvular incompetence of the lymphatics, a state of affairs which would also explain the appearance of "islands" of dye.

The stagnation of lymph in the edematous skin of the cardiac patients is not easily explained. In cardiac incompetence the venous pressure is generally greater than normal. Can the higher pressure in the veins at the point where thoracic duct lymph enters the blood be transmitted to the peripheral lymphatics and account for the stasis of lymph? Four tests were made on this point. Patients with cardiac failure and edema of the ankles and legs but not of the arms were so placed in bed that the wrists and ankles lay at the same level. In this position the effects of high venous pressure at the openings of the thoracic duct, acting to exert back pressure in the lymphatics, must have been the same in the channels draining both the upper and lower limbs. Nevertheless dye injections in the non-edematous arms resulted in the development of colored streamers with the rapidity and intensity seen in normal subjects, whereas dye introduced into the skin of the ankles gave rise to no streamers in the edematous legs. Lymph flow in the arms appeared to be normal whereas in the legs it was absent, a finding which would appear to rule out decisively the influence of back pressure to account for the stasis of lymph.

In contrast to the stagnation of lymph which obtains in the edematous legs of cardiac patients, there was found to be an increased lymph flow in the skin of patients with edema accompanying nephritis attended by a lowering of the plasma protein concentration. The patients were studied during periods of edema increase and diuresis. During the formation of edema there was in all of them a streamer formation, slightly greater than that observed in normal legs; and in all the onset of diuresis was accompanied by an extraordinary and intense streamer formation.

The following is a typical instance. A patient had been injected repeatedly over a period of weeks during which his edema slowly in-

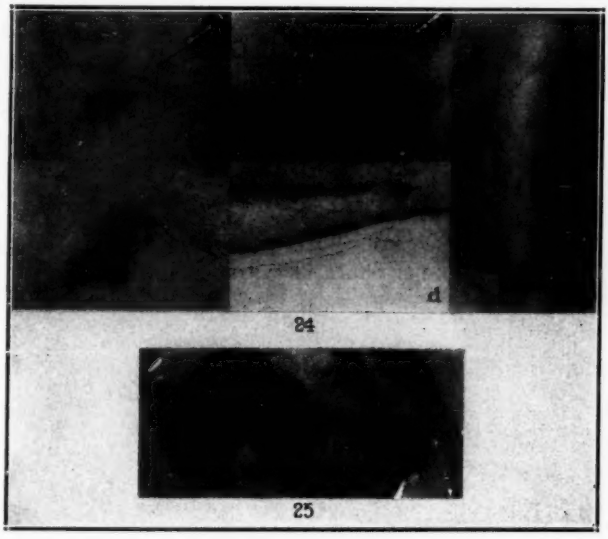

Fig. 24 *a-c*—Results of an intradermal dye injection in an edematous, nephritic patient during a period of diuresis. The injection required 55 seconds. Photographs *a*, *b* and *c* (natural size) were taken 17 and 51 seconds, and 1½ minutes, respectively, after beginning the injection. Fig. 24 *d* (1/12 natural size) and 24 *e* (1/11 natural size) were taken 15-18 minutes after the injection and show the intensity of the colored streamers, which by this time had extended above the knee.

Fig. 25—The displacement of dye-stained interstitial fluid by the intracutaneous pressure of a developing wheal. For details of the demonstration see the text. The wheal, which was evoked by a firm stroke, can be seen as a pale band lying transversely across the picture. Natural size.

creased. In the first few weeks the appearance of the lymphatics differed but little from normal and no photographs need be shown. As edema increased the lymphatic capillaries became wider than in normal legs, though not as wide as in patients with advanced cardiac edema. Fig. 24 *a*, *b* and *c* shows the appearance of the lymphatic capillaries at the height of his edema. By chance the patient had been injected upon the day that spontaneous diuresis commenced. Streamers formed within 3 to 4 minutes after the injection, which were far longer than any we had seen in normal legs even half an hour afterwards. Within 28 minutes intensely colored streamers extended from the ankle to Poupart's liga-

ment. Fig 24 *d* and *e* shows them 15 to 18 minutes after beginning the injection (which lasted only 55 seconds). In many other patients injected during diuresis, even more pronounced and more rapid streamer formation occurred. Such findings have never been obtained in normal skin.

From all this it seems certain that in heart disease the lymphatics fail in their function of fluid transport, adding to the edema. In nephritis on the other hand the lymphatics aid in the removal of the accumulated fluid.

Human Skin Lymphatics and the Defense of the Body Against Injury and Inflammation

We have studied, together with Hudack,[28] the fate of substances entering the lymphatics of human skin in regions of injury and inflammation, repeating many of the experiments made upon the mouse ear and adding new ones.

Permeability studies of lymphatic capillaries of living human skin showed that their walls behaved like semi-permeable membranes, as in the case of the mouse. Highly diffusible dyes passed through their walls more rapidly than poorly diffusible ones. An increase of the concentration of injected dye enhanced its passage through the lymphatic capillary wall but the addition of serum retarded it. In many experiments done under a binocular microscope, scratches were made with a fine sterile dissecting needle, so superficially that only the epithelium was removed, without tearing of skin venules or capillary tufts and without bleeding. Crystals of dyes of graded diffusibility were allowed to dissolve in the fluid transudate into these scratches. Soon the coloring matter appeared in the lymphatics draining the scratched region. The highly diffusible dyes passed into the lymphatics more rapidly than did the indiffusible ones. These findings showed not only that the lymphatic capillary wall behaved like a semi-permeable membrane but it served to test the permeability of the lymphatic capillary wall in the direction which interstitial fluid naturally takes to enter the lymph stream. Further, the experiments demonstrated how readily substances enter the lymphatics after injury to the skin.

Mild stimuli, of the sort met with constantly in everyday life, such as a stroke with a blunt instrument too slight to abrade the skin surface, exposure to heat of 53° C. for one minute, or to ultraviolet light, in-

crease the permeability of the lymphatic capillary wall, as evidenced by a more rapid escape of dye from the channels.

Sharply localized burns were made in human skin either by ultraviolet radiation or by heat. Lymphatic capillaries outside the burned areas were injected and carried colored fluid into the regions of injury. The lymphatics in the latter let dye through into the interstitial tissue far more readily than did those of normal skin.

Skin Lymphatics during Wheal Formation: The behavior of cutaneous lymphatics during the formation of wheals had not hitherto been investigated. If human skin which responds to stroking by wheal formation is submitted to a whealing stroke and the lymphatic capillaries of the region are immediately injected, they are found to be abnormally permeable during the latent period of about 2 minutes before the wheal appears. As it develops the lymphatics are emptied of their contents by the pressure of the wheal fluid and being compressed lose their effectiveness as drainage channels. If interstitial fluid already colored by dye is present it is displaced by the fluid which accumulates and forms the wheal. Since the latter remains uncolored, this fluid presumably is derived wholly from the blood.

The displacement of dye-stained interstitial fluid as whealing occurs is illustrated by Fig. 25. Two intradermal injections of an isotonic solution of a highly diffusible dye, patent blue V[28,37] had been made in the skin 4 hours before the photograph was taken. The introduction of this dye had produced a mild local edema[37,38] and the edema fluid had become colored with it. The skin was firmly stroked across the resulting colored patches and 3 minutes later the photograph was taken. The wheal appears as a broad pale band along the horizontal line of stroke. On both sides of it there can be made out a dark line of dye-stained fluid displaced by the wheal.

When histamine together with dye solution was injected into the lymphatic capillaries the latter became much more permeable during the period of whealing, as judged by the rapidity of dye escape from the channels. In several experiments histamine was pricked into a region of skin in which lymphatic capillaries had been injected with dye 2 minutes previously. As each wheal developed the pressure at its extending margin squeezed the colored solution along the lymphatics into normal skin beyond. Dye which had escaped into the interstitial tissue was pressed out of the whealing area, with result that the wheal became

surrounded by a darkly colored ring.

Sir Thomas Lewis[39] in his studies of the responses of blood vessels of the skin called attention to finger-like extensions of histamine wheals as indicative of a spread of the substance through the lymphatics, and described secondary wheal formation along the course of these vessels as result of its escape. With Hudack we have injected dye into the skin over fully formed but fresh histamine wheals.[28] The coloring matter passed into the lymphatics and each finger-like extension or "pseudopod," as they have been termed,[39] was observed to contain a dye-carrying lymphatic. During regression of the wheal the dye in the lymphatics paled rapidly, being carried away in the lymph stream.

The part played by the lymphatics in the formation and drainage from wheals has been further studied by Abramson and Engel.[40] On the basis of their findings and ours it is probable that a large histamine wheal forms, "not by diffusion of the histamine but by convection into the lymphatic capillaries and secondary escape into the interstitial tissues." There, part may act upon blood vessels, part may be taken up again by the lymphatics and escape at a new site to renew its activity upon blood vessels.

The Effect of Local Injections of Toxins and Bacterins: The inflammatory reactions of skin form the basis of many immunological tests; for example, Schick and Dick tests and the skin tests for allergy. In skin inflamed by injections of toxins or bacterins[28] the lymphatic capillaries become much more permeable than in normal skin. Dye which escapes secondarily into the interstitial spaces is removed in about one-fourth the time required to remove it from normal skin. For the sake of brevity the experiments which show these points will not be detailed here; we can only refer to published work.[28] Suffice it to say that these skin reactions cannot be considered as purely local.

Every Intradermal Injection Is in Part a Systemic Injection: That the lymphatics are involved in local infective processes is well known but the fact seems not to have been sufficiently recognized that noxious materials, whether toxic or bacterial, have immediate access to the lymphatics once the primary barrier of the epidermis is broken. If a sterile sharp needle was dipped into a dye solution or a suspension of dye particles and the skin lightly punctured with it, the dye solution or dye particles appeared in the lymphatics close to the puncture. Isotonic vital dye solutions placed upon a superficial scarification of the skin, like that

employed for clinical vaccination, too superficial to elicit bleeding, were taken up by the lymphatics and carried away. If a knife, dipped in a dye solution or a suspension of fine particulate matter, was used to cut the skin superficially, and the cut was then sucked, as one might suck a similar injury in everyday life, the pressure thus exerted upon the skin forced the dye or the particles which had entered the torn lymphatics several centimeters up the channels. The foreign material could not be squeezed back into the cut. The experiments showed that however slight the injury, colored particulate or diffusible matter punctured, scratched or injected into the skin found its way into the regional lymphatics. Further, as we have already described, severed lymphatics may remain open for a long time. As a result of all this the matter of local injection assumes greater importance in the light of the fact that intradermal injections are to a considerable extent intralymphatic; indeed every local injection is in reality a general one.

The Lymphatic System and Defense Against Infection

Since small particles of every sort (amongst them bacteria, poisons, viruses) can enter the lymphatics through every scratch or puncture, what is to prevent them from reaching the blood? It has long been known that lymph nodes act as filters for bacteria entering the lymph stream, but it is also kown that they are highly imperfect filters. The possibility suggested itself that the nodes might do more than act as mere filters, that they might play a part in the formation of antibodies.

A variety of experiments done with Hudack have brought out the fact that lymph nodes nearest the site of an intradermal injection of pathogenic bacteria form antibodies[41] before these appear in any noteworthy amount in the blood. Many other experiments have ruled out the possibility that the antibodies found in the regional lymph nodes had really been formed elsewhere in the body.

Proof of these statements reported at length in earlier papers[41,42] need not be detailed again. Here we will merely suggest the way in which one type of experiment was done. Killed cultures of agglutinin-forming bacteria were intradermally injected into one ear of large numbers of mice. Into the other ears of the mice Schick test toxin was injected. Daily thereafter, for some while, the serum, extracts from the cervical nodes of both sides, of nodes elsewhere in the body, of the liver, and of the spleen were tested for agglutinin content. After several days agglutinins

appeared first, in high concentration, in the extracts of the cervical nodes draining the ears injected with the agglutinin-forming bacteria. They were present too in the blood, in traces, but they were absent from the extract of the lymph nodes draining the ears injected with the Schick toxin and from the extracts of the other tissues. As the ears and the cervical lymph nodes on both sides were inflamed to the same extent, agglutinins formed elsewhere in the body and present in the blood would have had equal opportunity to be taken up by the cervical nodes of both sides, but they appeared only in the nodes of one side, that injected with the agglutinin-forming bacteria.

Each day the agglutinin content increased in the extracts of the nodes from the side injected with the agglutinin-forming bacteria and in the blood too, but not until a week later did they appear in the extracts of tissues taken elsewhere from the body.

Experiments of the same type, but made upon rabbits, were next undertaken with John G. Kidd[42] to learn whether the substances which neutralize viruses are also formed in the lymph nodes. It was found that the lymph nodes nearest to the site of invasion form the first antiviral substances neutralizing the virus.

Summary

The experiments here described provide a new conception of the lymphatic system. The lymphatics are very different vessels and far more important than had previously been supposed. They have been considered mere passive collecting channels and rather sparse at that, a view that came to be held because they are not rendered visible by ordinary means. Actually the lymphatics are so abundant in skin that the latter can nowhere be entered forcibly without tearing them, and further, when torn, they remain open. Flow in the smaller lymphatics, even in motionless tissues, is generally far more rapid than has previously been thought and forces have been found to account for it. The transport of foreign substances by way of the lymph is much more rapid than the volume of lymph flow would lead one to suspect, for often the channels are flat and ribbon-like and as result the flow of a very small amount of lymph may carry the foreign materials far through the channels.

The lymphatic capillaries possess walls that respond with remarkable rapidity to highly various stimuli and they are channels which take part

actively in the processes of fluid exchange. Influences that come within the realm of the normal—sunlight, slight warmth, a stroke that does not break the skin—all increase lymphatic permeability. Much of the usefulness of the lymphatics under this or that condition depends upon how permeable their walls are at the time. While they never become so permeable as to permit the immediate passage of particulate matter, nevertheless, at times, they cease to be walled off channels so far as physiological events are concerned.

About injuries and mild burns the permeability of the lymphatics increases enormously without actual loss of the anatomical continuity of their walls. Like the blood vessels, dye-containing lymphatics respond to injury by pouring coloring matter into the region of injury and all about it too. Of greater interest is the fact that the lymphatics regain their normal permeability earlier than the blood vessels do, and hence one may infer that through these channels the noxious products are first drained away and carried to the lymph nodes before reaching the body. Unlike the blood vessels the lymphatics may remain open long after they have been severed, and infectious agents can spread far through the body along their pathways.

The role of the cutaneous lymphatics in edema is of interest. In cardiac edema, perhaps because of extreme dilatation of the channels which renders their valves incompetent, the lymphatics fail in their function of fluid drainage, and so add to the disability. On the other hand, in the edema accompanying nephritis, the cutaneous lymphatics aid in the drainage of fluid from the skin and during the periods of diuresis there is an extraordinarily rapid lymph flow.

Finally, it is most noteworthy that every scratch and puncture, every injury that breaks the continuity of the skin, introduces foreign substances directly into the lymphatics. Every local injection is in reality a general one and as result of lymphatic drainage the regional lymph nodes play their part as the first line of defense, for in the nodes antibodies are first formed against both bacteria and viruses.

From all this it follows that what happens in the skin assumes greater importance now that it is apparent that injections into the skin are really injections into the lymphatic system and that the immunity against disease, conferred by preventative injections, even the reaction to the injection itself, is not merely a skin phenomenon but a generalized activity of the lymphatic system.

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DEATHS OF FELLOWS

ALTMAN, EMIL: 378 West End Avenue, New York City; born in Hungary, June 13, 1873; died in New York City September 11, 1942; graduated in medicine from the Columbia University College of Physicians and Surgeons, New York, in 1895; elected a Fellow of the Academy May 5, 1921.

Dr. Altman was chief medical examiner of the Medical Board of the Board of Education from 1924 to 1941, having joined the Medical Board in 1919, after serving on the school board from 1896 to 1914. He was consulting neuropsychiatrist to the Beth Israel Hospital, a Fellow of the American Medical Association, a member of the American Psychiatric Association, and a member of the State and County Medical Societies.

BROWN, WADE HAMPTON: Princeton, New Jersey; born in Sparta, Georgia, October 18, 1878; died at Rice Lake, Wisconsin, August 4, 1942; graduated in medicine from Johns Hopkins University School of Medicine, Baltimore, in 1907; elected a Fellow of the Academy, January 7, 1932.

Dr. Brown was instructor of pathology at the University of Virginia, 1907 to 1908; instructor of pathology from 1908 to 1910 and assistant professor from 1910 to 1911 at the University of Wisconsin, Madison; and professor of pathology at the University of North Carolina, Chapel Hill, from 1911 to 1913. That same year he joined the Rockefeller Institute for Medical Research, serving there as an associate member from 1914 to 1922, when he became a member of the scientific staff for medical research. He was a member of the Association of American Physicians, American Association of Pathologists and Bacteriologists, the American Society for Experimental Pathology, the Society for Experimental Biology and Medicine, the American Society for Pharmacology and Experimental Therapeutics, and the American Association for the Advancement of Science.

Dr. Brown was the contributor of more than seventy-five papers to medical journals. He specialized in the study of biology of syphilitic infections, and constitutional factors and physical environment in relation to heredity and disease.

FRAUENTHAL, HERMAN CLAY: 161 West 86 Street, New York City; born in Wilkes-Barre, Pennsylvania, September 15, 1866; died in New York City, August 23, 1942; graduated in medicine from Bellevue Hospital Medical College of New York City in 1897; elected a Fellow of the Academy October 6, 1904.

Dr. Frauenthal was one of the founders and consulting orthopedic surgeons to the Hospital for Joint Diseases, a Fellow of the American Medical Association, and a member of the State and County Medical Societies.

GEYELIN, HENRY RAWLE: 33 East 68 Street, New York City; born in Villa Nova, Pennsylvania, May 12, 1884; died in New York City, September 7, 1942; graduated in medicine from the University of Pennsylvania, School of Medicine, in 1909; elected a Fellow of the Academy January 3, 1929.

Dr. Geyelin was instructor of clinical pathology at the Columbia University College of Physicians and Surgeons from 1913 to 1916, associate in clinical pathology, 1916-1917, associate in medicine from 1917 to 1921, and since then assistant clinical professor of medicine; since 1921 he was associate attending physician at the Presbyterian Hospital, where he was formerly a Blumenthal fellow in medicine and assistant visiting physician; chief of the medical clinic of the Vanderbilt Clinic, 1918-1919; consulting physician to the Babies Hospital from 1923 to 1928, visiting physician from 1928 to 1932 and since 1933 associate attending physician; consultant specialist in diseases of metabolism at the U. S. Veterans' Hospital No. 81 from 1924 to 1933; and attending physician and a member of the medical board of the Doctors Hospital since 1929. He was a diplomate of the American Board of Internal Medicine, a member of the American Association for the Advancement of Science, the Society for Experimental Biology and Medicine, the Association of American Physicians, the American Clinical and Climatological Association, the American Society of Clinical Investigation and the Harvey Society, a Fellow of the American Medical Association, a Fellow of the American College of Physicians, and a member of the State and County Medical Societies.

TAYLOR, FIELDING LEWIS: 950 Fifth Avenue, New York City; born in Martinsville, Virginia, May 24, 1868; died in New York City, June 22, 1942; graduated in medicine from the University of Virginia, Department of Medicine, Charlottesville, in 1891; elected a Fellow of the Academy June 6, 1901.

Dr. Taylor was formerly an associate in medicine at the Cornell University Medical College and on the staff of the City Hospital and the Hudson Street Hospital. For many years he was consulting syphilologist to the New York Hospital. He was a Fellow of the American Medical Association and a member of the State and County Medical Societies.

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